A survey of pyrogenic carbon in Kootenay National Park burned soils, and its positive effect on the establishment of pine-fungal ectomycorrhizal symbiosis

by

Michelle Ruth Mausolf

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Abstract

Wildfire is a natural disturbance in Rocky Mountain forest landscapes. Fire plays an important role in maintaining stand structure, woody debris consumption, and soil nutrient cycling. Fire exclusion in these ecosystems has expanded forest cover, altered stand structures, and allowed accumulations of duff, litter, and organic materials at the soil surface. Wildfire management and prescribed burning are two strategies currently used in Canadian National Parks to restore fire to Rocky Mountain ecosystems. This thesis examines the long-term influence of wildfire and prescribed burning on soil pyrogenic carbon (PyC), organic matter quality, and microorganisms at fire sites in Kootenay National Park as well as the impact of PyC on mycorrhizal colonization of pine seedling roots in a lab-based experiment. Mycorrhizal fungi form mutualistic symbioses with vascular plants wherein nutrients from the soil are exchanged for photosynthetically derived carbohydrates. Mycorrhizal fungi in forest soils have been shown to increase tree survival in harsh conditions created after fire. Soil samples from LFH and mineral horizons were taken from transects on four sites including: wildfires that occurred in 1968 and 2003, a fireguard burned in 2003, and a prescribed burn conducted in 2008. PyC was quantified in soils using chromatography following oxidation, and organic matter quality was characterized using simultaneous thermal analysis. Prescribed burn mineral soil contained the lowest quantity of PyC of the sites studied. Prescribed burn soil also contained the highest total organic carbon, which had a greater influence on their organic matter quality, (thermal stability), than PyC. The microbial community in the prescribed fire soils was distinct from the wildfire and fireguard sites. Results of this survey demonstrate that a single prescribed burn was not adequate to replicate wildfire in stands with heavy fuel loading resulting from fire exclusion. To investigate the effect of PyC (simulating restoration with fire) on soil fungi that form ectomycorrhizal symbioses with pine, microcosms containing *Pinus banksiana* and *Suillus tomentosus* were amended with biochar quantities representative of high and low severity fire. Biochar had no effect on the growth of non-mycorrhizal pine, and a negative effect on the growth of non-mycorrhizal *S. tomentosus*. However, in microcosms amended with biochar consistent with moderate-low severity wildfires, growth of pine seedlings and fungal hyphae was higher than in microcosms without biochar amendment. These results demonstrate that low to moderate severity prescribed fires may be beneficial to establishing mycorrhizal pine seedlings and play an important role in fire restoration in Rocky Mountain ecosystems.

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Chapter 1: Literature Review

The health of many Rocky Mountain ecosystems is in decline because of fire exclusion (Keane et al. 2003). Fire is a dominant disturbance and important ecological process in Rocky Mountain landscapes. Nutrient recycling, maintenance of diversity, insect population control, biomass reduction, and maintenance of biological and biogeochemical processes are all ecosystem services provided by fire (Agee 1993, Goldammer and Crutzen 1993, Mutch 1994). Historical fire regimes in the Rocky Mountain region included frequent, mixed low-to-moderate severity fires that created forest structural complexity with fuel heterogeneity and unburned refugia (Knapp and Keeley 2006, Ryan et al. 2013). Mixed-severity regimes produced landscape fuel heterogeneity by creating forests containing multi-aged stands of fire-dependent species, including large, old fire-resistant trees important as wildlife habitat (McClelland et al. 1979). Fire is unique as a disturbance in that it changes the physico-chemical and biological soil environment though heating and oxidation, and also creates new sources of physico-chemical and biological inputs into the soil system (Hart et al. 2005). Pyrogenic carbon (PyC) compounds left behind by fire have a long term influence on soil quality, microbial activity, and vegetation (Zackrisson et al. 1996).

After 1945, policies mandating fire suppression and advances in suppression tactics and equipment resulted in increased exclusion of fire from Canadian Rocky Mountain landscapes (Woodley 1995). From 1945 to 1979, Parks Canada and the Alberta and British Columbia provincial governments enacted comprehensive suppression plans throughout the Rocky Mountains. Government support, public messaging campaigns, and agricultural and recreational use of the region contributed to land management policies that saw fire suppression as the most desirable strategy for public lands (Mutch 1995). Canadian National Parks policy was changed in

1979 to allow for fire and other natural ecosystem processes to occur under limited conditions directed by plans for park vegetation and fire management (Hawkes 1990). Nonetheless, 30 years of fire suppression followed by limited burning since the 1979 policy change has resulted in numerous landscape level changes from historical conditions that are shared across the Rocky Mountain region (Keane et al. 2003). The effects of fire suppression across the Rocky Mountains can be summarized in three categories; altered landscape-scale fuel load characteristics, changed vegetation structure, and impacts on below ground processes and biota.

1.1 Altered Fuel Characteristics

Fuel characteristics altered by fire exclusion include increased vertical ‘ladder’ fuels, higher surface fuel load, and greater litter and duff depths (Brown 1985, Debano 1991). In fire excluded forests, tree regeneration increased, and open areas became forested (Benedict 1930), both effects that increased forest continuity, numbers of small trees, and surface woody debris fuel loads (Skinner 1995). Extreme wildfire behavior and uncharacteristically severe fires have been attributed to the effects of fire exclusion on fuel characteristics. For example, Agee and Skinner (2005) found that in drier portions of the Rocky Mountains, greater fuel continuity and the influx of conifer saplings contributed to higher fire intensity and severity, and an increased probability of crown fires. Fire severity describes the impact of fire on an ecosystem, and differs from fire intensity, which specifically describes the heat or energy output from a fire (Keane et al. 2003). Fire severity can be described with three general classes related to depth of burn and tree mortality; low or nonlethal, mixed-severity, which results in a mosaic of burn effects and tree ages, and high-severity or stand-replacing fire, which results in uniform tree age (Morgan et al. 1996). The current combination of suppression tactics and greater fuel load has affected fire behavior in that wildfires tend to be less frequent and more severe than those that occurred prior to 1900 (Morgan et al. 1996).

The following principles have been suggested to guide forest management to lower the likelihood of severe wildfires and mitigate the altering effects of fire suppression on fuel characteristics; reduction of surface fuels, increasing height to live crown, decreasing crown density, and retaining large trees of fire-resistant species (Agee and Skinner 2005). Prescribed burning is an effective tool that fits within that guidance to reduce surface fuels, as well as to reduce ladder fuels and scorch lower tree crowns, thereby increasing height to live crown (van Wagtendonk 1996).

1.2 Altered Vegetation Structure

The current fuel connectivity and abundance observed in the Rocky Mountains is closely related to regional vegetation structure. As a consequence of fire exclusion, regional forest composition has shifted from a landscape stand-age mosaic of single-layer canopied forests composed of shade-intolerant tree species, to a denser, multiple-layer canopy composed of shade-tolerant species, with greater landscape-level forest homogeneity (Veblen and Lorenz 1991, Mutch et al. 1993, Steele 1994, Quigley and Arbelbide 1997). Multi-layered canopies and shade-tolerant shrubby ladder fuels increase fire spread from surface fuels into tree crowns in a process known as torching. Torching is a characteristic behavior of severe wildfires, and the initiation behavior of crown fires (Van Wagner 1977, Agee et al. 2000). Forest management to mitigate altered vegetation structure should include reduction of canopy density, a principle that aims to increase forest resilience to wildfire by reducing crown fire behavior (Agee and Skinner 2005). Unfortunately, prescribed fire, while effective at increasing height to crown which can reduce torching, is generally less effective at reducing canopy bulk density, as prescribed fires intense enough to kill larger trees are more likely to exceed desired severity thresholds (Agee and Skinner 2005, Miller and Urban 2000). Further, single prescribed burns in forests with dense, multi-layered canopies tend to leave behind high surface fuel loads of standing dead trees (Agee 2003). A multi-year program of repeated burning that resembles a natural mixed severity fire regime is necessary to effectively consume these high loads of standing dead trees and reduce stand fire hazard in regions with altered vegetation structure from fire exclusion (Allen et al. 2002, McCandliss 2002).

1.3 Altered Soil Characteristics

The impact of fire on soil is due to the components of fire severity; fire intensity, the rate at which fire produces thermal energy, and duration, however, duration is the component of fire severity that causes the greatest below ground damage (Certini 2005). Combustion and heat transfer create steep temperature gradients in soils, where the depth trend of temperature depends on fuel bed thickness and packing ratio, and both fuel and soil moisture (Hartford and Frandsen 1992, Gillon et al. 1995, Campbell et al. 1995). Heat in moist soil is transported faster and penetrates deeper, but, latent heat of vaporization prevents soil temperature from exceeding 95 °C until water completely vaporizes (Campbell et al. 1994). Without sufficient duration, an intense but fast moving fire may not transfer much heat down through soil horizons, therefore having less severe impacts on soils than slower moving smoldering combustion (Certini 2005).

Fire alters soil physico-chemical and biological properties through combustion-mediated losses and oxidation in the short term, but also creates new physico-chemical inputs in the form of pyrogenically altered carbon compounds (Hart et al. 2005). Soil heating raises pH ephemerally as a result of organic acid denaturation, with significant increases coinciding with soil temperatures above 450 °C, and complete combustion of fuels leading to the release of base cations (Ca^+, Mg^+, K^+, Na^+) (Arocena and Opio 2003). Fire leads to immediate losses of soil organic matter, (SOM), ranging from substantial to complete loss to combustion depending on temperature (Giovannini et al. 1988), however increases in SOM have been reported as a positive long term effect of fire (Certini 2005, Johnson and Curtis 2001, Gonzalez-Perez et al. 2004). A meta-analysis by Johnson and Curtis (2001) attributed post-fire increases in SOM to 1) incorporation of unburnt organic residues into mineral soil, where they were protected from biochemical decomposition, 2) frequent entrance of N-fixing bacterial species into burnt areas that significantly enhance soil carbon sequestration, and 3) transformation of fresh organic materials into recalcitrant forms of pyrogenic carbon (PyC). A literature review by Gonzalez-Perez et al. (2004) summarized the main effects of fire on SOM that contribute to its increased recalcitrance as PyC; 1) aromatization of sugars and lipids, 2) general removal of external oxygen groups, which yields materials with reduced solubility, 3) formation of heterocyclic N compounds, and 4) macromolecular condensation of humic substances. Soil biological properties altered by fire include microbial biomass and community composition (Certini 2005). The impact of fire on these biological properties depends on soil moisture, with the largest biomass reductions associated with the moistest soils (Choromanska and DeLuca 2002). Long term recovery of microbial biomass and community composition is directly influenced by the strong interrelationships between plants and soil microorganisms (Hart et al. 2005).

Altered soil dynamics specifically resulting from fire exclusion include decreased nutrient availability, increased pore space and water holding capacity related to accumulation of soil organic matter, and lower soil temperatures (Debano 1991, Martin and Sapsis 1992). Fire exclusion allows the accumulation of duff, litter, and soil organic matter, and removes the regular input of PyC compounds into soils (Keane et al. 2003). These altered soil properties shape the microorganism community and its interactions with plants in the long term after fire. For example, in coniferous forests, fire exclusion may lead to late secondary succession under which microbial activity declines. Wardle et al. (1997) attributed this observation to limitations in microbial activity and organic matter decomposition imposed by excessive concentrations of phenols not adsorbed and inactivated by freshly charred materials. Further, after extended fire exclusion, when a fire does occur, the combustion of thick accumulation of litter and soil organic matter result in deep soil heating which causes high plant propagule and microorganism mortality (Hungerford et al. 1991). Fire exclusion decreases nutrient availability by interrupting or limiting decomposition to bio-available forms. While there is abundant N in the large amounts of organic matter accumulated as a result of fire exclusion, only a small portion of this N is made available to plants from decomposition by soil organisms (Waring and Running 1998). The combustion process releases some of the sequestered N and makes it bio-available as ammonium as it condenses on lower soil layers. Combustion also converts the organic P pool to bio-available orthophosphate (Cade-Menun et al. 2000).

1.4 The effect of Pyrogenic Carbon and its Legacy

Pyrogenic carbon (PyC) describes a range, or continuum, of structures from charred plant materials, charcoal, soot, and its most condensed form, graphite (Seiler and Crutzen 1980). PyC is incorporated into soils after fire and remains a component of soil ecosystems long after fire due to its resistance to chemical and physical degradation (Schmidt and Noack 2000). PyC can make up significant fractions of soil organic matter in frequently burned ecosystems and contributes to the thermal stability of soil organic matter (Haumaier and Zech 1995, Leifeld 2007). Variation in fire intensity, a controlling element of fire severity (Keane et al. 2003), relates to differing amounts of PyC produced and deposited on the soil (Santin et al. 2016). For example, a high intensity wildfire in a conifer dominated forest deposited 6400 kg PyC ha^{-1} (Tinker and Knight 2000), whereas fires in similar conifer forests under lower fuel load or that burn with lower intensity were reported to produce from 984 – 2074 kg ha^{-1} (Zackrisson et al. 1996), or even less than 500 kg ha^{-1} (Zackrisson et al. 1996, Lynch et al. 2004). The degree to which fire intensity and severity translate into PyC generation is governed by interacting factors related to intrinsic fuel properties (e.g., material density and chemical composition), extrinsic forest and fuel properties (e.g., stand structure, moisture and load), and burning conditions (e.g., weather and duration) (Brewer et al. 2013, Santin et al. 2016).

Pyrogenic carbon has numerous effects on soil physico-chemical properties including nutrient availability (Deb et al. 2016, Laird et al. 2010), and soil organic matter (SOM) (Sanchez-Garcia et al. 2015). Pyrogenic carbon may influence nutrient availability and SOM stability through a priming effect with dual impacts, one on SOM stability, and second, on soil microbial activity. For example, PyC has been shown to speed up nitrification from SOM (Sanchez-Garcia et al. 2015), and PyC may promote P mobilization by stimulating soil microbial activity (Deb et al. 2015). Nutrient availability may also be increased by a reduction in leaching caused by a PyC-mediated increase in soil water retention (Laird et al. 2010). Oxidation of organic carbon compounds during combustion increases soil nutrient and water holding capacity by exposing carboxylic acids on the PyC aromatic molecular backbone (Glaser et al. 2002). Carboxylic acids exposed on the PyC molecular backbone also enable PyC to function as an effective sorptive surface for allelochemicals and other organic compounds in soil (Zackrisson et al. 1996).

Pyrogenic carbon has been shown to promote microbial growth and activity, and change microbial community composition (Zackrisson et al. 1996, Wardle et al. 1998, Pietikainen et al.

2000). Substrate induced respiration (SIR) has been used to show that PyC addition increases bacterial abundance across communities generally (Wardle et al. 2008, Kolb et al. 2009), as well as having an influence on specific groups of microorganisms. For example, PyC may play a role in maintaining the activity of nitrifying bacteria in late successional boreal forests (DeLuca et al. 2002). In addition to microbes, PyC also affects mycorrhizal fungi, organisms that form symbioses with roots of most conifers found in these landscapes. Mycorrhizal fungi in outdoor field soil experiments responded more positively to PyC additions than to other types of organic matter (Harvey et al. 1976, Ishii and Kadoya 1994), and PyC addition to forest soils has been shown to enhance lodgepole pine (*Pinus contorta*) seedling growth and the formation of ectomycorrhizas (Robertson et al. 2012). However, other studies report negative effects on ectomycorrhizas attributed to the effects of PyC on bio-available P, N, and carbon (Gaur and Adholeya 2000, Wallstedt et al. 2002).

1.5 Modifying Parks Policy on Wildfire Burning

Recognizing the necessity of wildfire for ecosystem health in recent years, land managers in the mountain National Parks adopted "let it burn" and prescribed burn policies over total suppression to restore fire to this ecosystem (Hawkes 1990). The majority of National Park hectares burned annually are the product of a small number of wildfires in remote areas that are allowed to burn or are not aggressively suppressed (Taylor 1998). Wildfire management has the potential to return fire to large areas, and to generate ecosystem heterogeneity through natural within-fire variation in fire intensity and severity (Collins et al. 2009, Ryan et al. 2013). However, the effects of fire exclusion, specifically, the loss of landscape fuel variability and fuel loads that exceed historical values, raise concerns about the potential high severity of wildfires in long unburned areas (Ryan and Frandsen 1991). A high severity wildfire can have negative outcomes for forest ecosystem recovery including widespread stand mortality, loss of soil seedbed, and burn depths to mineral soils; conditions that slow regeneration and rely on ecosystem resilience for recovery (Mutch 1994, Keane et al. 2003).

Along with wildfire management, prescription burning is used as a part of the Parks Canada strategy to return fire to the Rocky Mountain landscape (Weber and Taylor 1992). Prescription burning has the capacity to mitigate severe effects from later wildfires and limit extreme wildfire behavior, while restoring fire-dependent ecological processes (Cochrane et al. 2012). Prescription burning prior to wildfire may attenuate the effects of fire on soil and predispose the microbial community to the damaging effects of soil heating, which limits biomass loss to mortality (Choromanska and DeLuca 2001). These protective and beneficial effects of prescribed burning, in addition to a reduction in the likelihood of crown fires can last for a period of 5 years or longer (McCaw 2013). However, maintaining control of prescription fires is a primary concern, so low severity burning is often the objective (Ryan et al. 2013). To increase the likelihood of a low severity burn, many prescription fires are conducted in cooler seasons and under higher moisture conditions than are usually present during wildfires (Knapp et al. 2009). In addition to burning with lower severity and under wetter and cooler seasonal climatic conditions, prescription burns often have homogenous effects relative to wildfires because common ignition patterns, linear strips for example, are designed to homogenize fire behavior for easier control (Knapp and Keeley 2006).

The burn characteristics typical of prescription fires raise concerns about the effectiveness of prescription burning to act as a surrogate for wildfire in Rocky Mountain ecosystems (Ryan et al. 2013). Under typical low intensity burning conditions combustion efficiency is generally low (Certini 2005), so only a small part of surface fuel is exposed to thermal degradation, which can result in overall little PyC production (Graca et al. 1999, Schmidt and Noack 2000). In contrast, high intensity fires have a higher efficiency (Campbell et al. 2007) and affect a greater proportion of the fuel available. As a result, the quantity of PyC produced in a low-intensity prescribed fire may not be representative of PyC produced during wildfire, which is often more intense (Santin et al. 2016). Given the differences in burn conditions associated with wildfire and prescribed burning, it is reasonable to expect different resulting severities and outcomes on soil properties and microorganisms.

1.6 Research Objectives and Specific Questions

The overall objective of this thesis was to evaluate the differing effects of wildfire management and prescription burning on deposition of PyC in soil, and the resulting legacy effects on organic matter quality and soil microorganisms, including ectomycorrhizal fungi. Towards addressing this objective, I quantified PyC at a historical wildfire (47 years since fire), modern wildfire (12 years since fire), and at a fireguard (12 years since fire) and prescribed burn (7 years since fire). The comparison of the fireguard and prescribed burn allowed me to examine the effects of seasonality and high fuel load on PyC deposition during fires with anthropogenic ignition. My research investigated the influence of fire type on soil PyC quantity and tested the role of PyC in soil organic matter thermal stability. The soil microbial community at each burn site was characterized using community-level physiological profiling with the objective of relating microbial community function to fire restoration strategy, soil PyC content, and organic matter quality. To investigate the effect of fire restoration on forest soil fungi, ectomycorrhizal jack pine was grown with amounts of charcoal similar to those found at the fireguard and wildfire sites.

Specific research questions included: 1. What were the long term belowground impacts of prescribed burning and fireguard burning compared to wildfire, 2. Does PyC stimulate ectomycorrhizal colonization and development on pine, as a way to stimulate seedling growth for restoration.

Chapter 2: Pyrogenic carbon and microbial community characteristics in burned soils of Kootenay National Park

2.1 Introduction

The mountain national parks are made up of seven contiguous national and provincial protected areas covering 21,900 km^2 along 310 km of the Alberta/British Columbia provincial border. Four parks lie on the east side of the provincial border, in Alberta, and comprise 18,842 km^2 of the protected area. Parks west of the Alberta border include Yoho and Kootenay National Parks, and Mount Assiniboine Provincial Park; together they comprise 3,068 km^2. Kootenay National Park, established in 1920, covers 1400 km^2 in southeastern British Columbia.

Due to orographic effects on precipitation and lightning activity in the region, the eastern and western National Parks have different fire regimes. Fires in the eastern Parks, Jasper and Banff, are more likely to be ignited by humans, while fires in western Parks, Yoho and Kootenay, are more likely to be ignited by lightning (Van Wagner et al. 2006). Prior to 1840, tree ring and stand age-class data support a 90 – 100 year fire cycle in KNP, however, a declining burning rate, and erratic data, point to a non-equilibrium state in Park forests as of year 2000 (Van Wagner et al. 2006). Fire exclusion from large wilderness landscapes has led to greater stand density with increased basal area, and uniformity in age and composition, while decreasing biodiversity in undergrowth species (Arno et al. 1993, Keane and Arno 1993, Arno et al. 1997).

Charred biomass of various forms and degrees of combustion is the byproduct of fire, regardless of its source of ignition (wildfire or anthropogenic). Pyrogenic carbon (PyC) describes a range, or continuum, of condensed organic structures from charred plant materials, charcoal, soot and graphite (Seiler and Crutzen 1980). Fused aromatic rings in varying cluster sizes, with or without other elements (N, O) and functional groups, are characteristic chemical components of PyC (Preston and Schmidt 2006). PyC degrades very slowly in Boreal climates (Schmidt and Noack, 2000), which are similar to the Rocky Mountains, so it could be seen as long lasting evidence of fire activity in a particular area. Little previous work has quantified soil PyC in Rocky Mountain forests that have been affected by fire suppression, nor has the effects of PyC on microbial communities been characterized in this ecosystem. Fire suppression in Rocky Mountain forests has been associated with cascading negative effects including altered forest structures, loss of diversity and reduction in soil nutrient cycling (Keane et al. 2003). The fires that do occur in forests affected by years of suppression are hypothesized to burn with greater severity (Ryan et al. 2013).

Quantification of soil PyC may be useful for gaining insight into local fire history; fire frequency and burn severity. Soil PyC can be quantified based on visual detection of physical particles (Zackrisson et al. 1996, Belanger et al. 2004), or by various chemical methodologies (Preston and Schmidt 2006). Quantification by visual detection is based on particle size, involving hand-sorting from humus or soil cores, followed by sieving into size classes. Multiple techniques exist to quantify PyC based on its chemical properties, most of which rely on oxidation as the mechanism of action, or are based on quantification of molecular markers released during analysis (Preston and Schmidt 2006). A major problem in quantifying PyC is that it is not a single entity, rather it exists on a physicochemical continuum based on molecule condensation or aromaticity, ranging from large pieces of charred biomass, (1 – 100 µm), to submicron soot particles, (930 – 40 nm) (Hedges et al. 2000, Masiello 2004). Additionally, different quantification techniques may produce different results, because a single technique generally only captures a subset of the continuum, rather than all forms of thermally altered carbon (Hammes et al. 2007).

Considering these problems, I estimated the quantity and physicochemical condition (aromaticity) of soil PyC using two methods. First, to estimate soil PyC content, I quantified molecular markers for PyC, benzene polycarboxylic acids (BPCAs), which are released after condensed aromatic ring structures are oxidized by nitric acid. Second, I characterized the aromaticity of soil PyC by analyzing its thermal stability. The thermal stability of a compound refers to its resistance to degradation under high temperatures. Together, these analyses may be able to provide some insight into local fire frequency, fire behavior, and forest conditions at the time of burning, as these factor influence soil organic matter quality, and PyC quantity and thermal stability.

Soil heating and PyC abundance influence the microbial community in burned soils. Heat has immediate lethal effects on soil microbes, but, more relevant to this study, also has persistent indirect effects on survival and recolonization by soil microbes (Certini 2005). Soil heating indirectly affects soil organisms through reduction and modification of organic substrates, and removal of sources of organic residues (Bissett and Parkinson 1980, Monleon and Cromack 1996). Charcoal in soil has been shown to alter microbial communication by interfering with intercellular signaling molecules, like acyl-homoserine lactones, through physical sorption on its exterior surface, and through altered pH mediated decreases in signal bioavailability (Masiello et al. 2013, Gao et al. 2016). Community-level physiological profiling (CLPP) assesses utilization of a single carbon source substrate to characterize the capability of heterotrophic microbes in environmental samples (Leckie 2005). Patterns of utilization of various single carbon source molecules have been effective at distinguishing physiologic differences in microbial communities (Garland 1997).

As understanding of the negative effects of fire exclusion and acceptance of the role of fire in mountain ecosystems increased, Parks Canada's land management policy has moved away from full suppression of wildfires (Hawkes 1990). Parks Canada currently uses prescribed fire and strategic management of wildfires to return fire to the mountain landscape as the dominant ecosystem disturbance (Cochrane et al. 2012). Burning can be used to eliminate fuels ahead of an active wildfire front, with the objective of stopping the spread of the wildfire past the burned unit, which functions as a fire break or fire guard (Schullery 1989). A challenge faced by land-managers conducting prescribed burning is the primary concern of maintaining control of the fire, which favors cooler and wetter early season spring or fall burns at lower intensity (Ryan et al. 2013). Therefore, prescribed burning and wildfire or fire-guard burning can differ in fire weather indices, (temperature, relative humidity, fuel build-up, etc.) which can lead to distinct fire behavior and severity (Gray and Kubian 2010).

I collected soil from four Kootenay National Park (KNP) burn sites; two wildfires and two prescribed fires. These sites occur within close proximity to each other, providing a unique opportunity to compare soil PyC and microbial communities following different fires in similar vegetation communities with shared climatic conditions. The objective of this study was to use soil analysis techniques to compare the soil properties created by wildfire to those created by prescribed burning. This study quantifies soil PyC and profiles microbial community function at these sites and may facilitate a better understanding of the local effectiveness of prescribed fire as a proxy for wildfire on long-term ecosystem function.

2.2 Methods

2.2.1 Study Areas

During the fire season, May – October, the daily average temperature in Kootenay National Park (KNP) is between 2.6 ºC and 8.4 ºC. Extreme daytime maximums in July and August are 35.0 – 36.5 ºC. The region receives a monthly average of 52.5 mm of precipitation during the fire season, during which June is the wettest month (Canadian Climate Normals 1981-2010 Station Data, KTNY CRSG). Montane/subalpine soils are most commonly Brunisols and Podzols, with Regosols occurring where slope gradient is enough to interrupt horizon development (Coen et al. 1977). Brunisolic Bm horizons typically have a bulk density of 1.3 g cm^{-3} (Coen et al. 1977).

2.2.1.1 Vermilion Pass fire sites

The topography created by Mt. Whymper and the surrounding peaks, including Storm Mountain and Stanley Peak, in the north end of KNP create the Vermilion Pass, separating KNP from Banff National Park. The Vermilion Pass saw wildfire activity in 1968 and 2003, and a burned fireguard was created in 2003 as well. On July 9, 1968, a lightning strike ignited the forest on the slope of Mt. Whymper. The fire burned 2950 ha of Engelmann spruce and subalpine fir forest over three days before being extinguished by rain. Since the Vermilion Pass Burn in 1968, the predominantly lodgepole pine and Engelmann spruce forest had been left to recover naturally. In 2003, lightning ignited five fires in the Vermilion Pass that burned a collective 17000 ha. The fires spread northward toward the Bow Valley, prompting the construction of the Vermilion Pass Fireguard which used a combination of hand torching and heavy machine blading up-slope to eliminate fuels ahead of the wildfire front. The fireguard consisted of a burned area and a 40 m wide line where heavy machinery was used to remove surface organic matter on both sides of the Vermilion Pass (“The Vermilion Pass fireguard”, 2017). Figure 2.1 provides an aerial overview of the Vermilion Pass fire sites.

2.2.1.2 Prescription burn – Mitchell Ridge

The 2008 prescribed burn on Mitchell Ridge was carried out as part of an ecological restoration objective in response to a landscape-level outbreak of bark beetles attacking lodgepole pine. In response to the unprecedented bark beetle epidemic, Parks Canada used aggressive, proactive landscape-scale prescribed burning (Gray and Kubian 2010). Mitchell Ridge is west/ south west facing and located in the south corner of KNP. The 4000 ha burn unit experienced bark beetle infestations in the early 1980s and again in 2005, leading to high levels of surface fuel build-up. The prescribed burn was carried out on May 31, 2008 and resulted in lodgepole pine mortality in approximately 50% of the unit (Gray and Kubian 2010).

2.2.2 Soil Sampling

Soil sampling at all sites was carried out in three latitudinal transects. Transects were spaced 10 m apart, horizontally across the slope. Soil samples were collected every 10 meters for 100 meters and composited at each transect. Horizons were collected and composited separately; A horizons were observed to be approximately 4 cm thick, and B horizons were sampled to a depth of 15 cm. Color and texture were the primary diagnostic features used to differentiate horizons in the field. Depth measurements and samples were collected using a Hori knife with a dished blade, depth markings, one serrated edge and one sharpened edge. Samples were stored in ice packed coolers after collection, and during transport to the University of Alberta. Samples were frozen until laboratory analysis. This sampling design allowed characterization of a small area within each burn site.

2.2.4 Soil Organic Matter Quality

2.2.4.1 Quantification of benzene polycarboxylic acids

Benzene polycarboxylic acids range in structure from single to full (six member) conjugation of the benzene ring with carboxyl groups, however previous work shows that the sum of BPCAs with three to six carboxyl groups is a sensitive measure of soil PyC content (Glaser et al. 1998). This method has been shown to yield conservative estimates of PyC, and is suited to recovery of pyrogenic carbon in solution (Hammes et al. 2007), meaning it allows quantification of PyC particles smaller than those recovered by sieving into size classes. However, the BPCA method is a complex, multi-step procedure that is highly sensitive to operating conditions, resulting in low inter-laboratory reproducibility, with reported 2 – 12 factor variations and inconsistencies (Preston and Schmidt 2006). Pyrogenic carbon quantities estimated via this technique for commercially available soot and char were lower than expected, suggesting that the BPCA method does not recover the largest, or the most condensed PyC continuum components, rather, that the BPCA method most effectively detects carbon in larger aromatic clusters (Schmidt et al. 2001). For this reason, estimations of PyC in soils based on the BPCA method are usually reported using a multiplication factor, 2.27, when converting BPCAs to PyC (Glaser et al. 1998, Brodowski et al. 2005).

Benzene polycarboxylic acids (BPCA) were extracted from soils using nitric acid to break down PyC polymers into BPCA monomers using methodology adapted from Wiedemeier et al. (2013). Soil samples were digested with an oxidizing solution of 65% nitric acid and hydrogen peroxide. Following digestion, BPCA sample product eluates were freeze-dried, then re-dissolved in 1:1 ultrapure water: methanol and passed through a C-18 SPE cartridge to separate polar and non-polar compounds. Following separation, samples were air-dried, diluted, then filtered through a 0.22 µm cellulose syringe filter and frozen again prior to HPLC processing.

BPCAs were quantified using HPLC to estimate PyC in soil samples (Wiedemeier et al. 2013). Samples were analyzed by reverse-phase HPLC with a 240-nm detection wavelength, 16 ºC column temperature, 1.0 µL injection volume (Agilent Technologies, 1100 Series, Santa Clara, CA, USA). A PowerShell 120 EC-18; 4.6 mm x150 mm, 4 µm particle size column was used for analysis. Target BPCAs were B6CA, B5CA, 1,2,4,5-B4CA, 1,3,5-B3CA, 1,2,4-B3CA, and 1,2,3-B3CA (Appendix 1). These structures were chosen as targets because BPCAs with less than three carboxyl substituents may not be able to resist the severe oxidation conditions used during this analysis technique (Glaser et al. 1998). The sum of the six BPCAs was taken as a representation of pyrogenic carbon content and reported as PyC g/Kg soil. Pyrogenic carbon was estimated as 2.27 times the cumulative BPCAs recovered from the soils (Glaser et al. 1998, Brodowski et al. 2005).

During development of the BPCA method, Glaser et al. (1998) found that increased sample char time led to an increased yield of each BPCA, but that the relative contribution of single BPCAs to the total yield did not differ significantly with increasing charring time. Therefore, the assessment of the contributions of single BPCAs to the total yield found in the current study could provide insight into fire residence time and show differences between the studied fires.

Pyrogenic carbon quantities were scaled up to kg/ha to place the quantities presented in the current study at a landscape level. Calculations were made using a general Bm horizon bulk density of 1.3 g soil/cm^3 for Brunisolic soils found in the Kootenay National Park region (Coen et al. 1977). Individual site bulk density was not able to be accurately calculated due to the abundance of shallow buried rocks encountered while sampling. Calculations are based on PyC found in g/kg soil in B horizons, to a depth of 15 cm.

2.2.4.3 Soil organic matter determination

The organic matter, OM, content of the soil samples was determined thermogravimetric analysis (TG; STA 6000, Perkin Elmer). The change in sample weight, or loss on ignition, represented the organic matter content that was present (Robertson 2011). The following equation was used to calculate % organic matter of each soil sample:

$$\%OM = \frac{Pre\text{−}ignition\ weight\ (g) - Post\text{−}ignition\ weight\ (g)}{Pre\text{−}ignition\ weight\ (g)} * 100$$

Total organic carbon was estimated as 0.588 of sample organic matter as calculated from loss of ignition during thermogravimetric analysis (Freytag et al. 1985).

2.2.4.4 Thermal analysis

Simultaneous thermal analysis (STA) combines the application of thermogravimetry (TGA), where the mass of a sample is measured over time as temperature increases, with differential scanning calorimetry (DSC), whereby the amount of energy as heat required to increase the temperature of a sample is measured as a function of temperature (Oneill 1964). Simultaneous thermal analysis can provide information about the thermal stability of soil organic matter (SOM) based on decomposition (mass loss) and internal degree of molecular order/crystallinity (Emadi et al. 2005). The degree of crystallinity or aromaticity of organic carbon is postulated to be the single most important factor determining oxidative thermal stability of carbonaceous materials (Leifeld 2007). Soil heating during fire usually leads to the loss of the most labile components of SOM, like carbohydrates and lipids, and to the generation of aromatic substances like PyC (Knicker 2007, Fernandez et al. 2011). Here, I characterized the degree of SOM aromaticity by analyzing heat of combustion, Q, (J g^{-1} soil) over three temperature regions along the exothermic range of the DSC curve. Heat of combustion evolved at the cool end of the exothermic DSC curve range was attributed to labile organic matter, while Q evolution at the highest temperatures was interpreted as an indicative of highly recalcitrant PyC in the burned soils.

Soil thermal stability was measured on dried soil samples using Differential Scanning Calorimetry (DSC; STA 6000, Perkin Elmer). Approximately 20 mg of each sample was loaded in an open ceramic pan under an oxidative atmosphere (flow rate: 20 mL min-1 Oxygen gas and 80 mL min-1 Nitrogen gas) at a scanning rate of 20 °C min^{-1}. The heat of combustion (Q in J g^{-1}) was determined by integrating the DSC curve over the exothermic region (150° - 550°C). Within this region, the area on the DSC curve was divided into three groups based on their degree of resistance to oxidation: (1) labile organic matter (150°C-375°C), (2) recalcitrant organic matter (375°C-475°C) and (3) highly-recalcitrant organic matter (475°C-550°C). These regions were modified from Merino et al. (2014); Merino et al. (2015); and Merino et al. (2016).

2.2.5 Community-level Physiological Profile

Community-level physiological profiles (CLPP) were assessed to investigate the diversity of microbial function across the sites. Community-level physiological profiles were assessed directly on soils by measuring substrate-induced respiration following addition of a single carbon source substrate (Degens and Harris 1997). In this way, CLPP characterized the heterotrophic microbial community in soils (Leckie 2005). CLPP was adapted from the multi-substrate induced respiration microtiter plate based procedure described by Campbell et al. (2003). Sieved soil samples were incubated with roughly 60% of field capacity water (measured by pressure plate (Richards and Fireman 1943, Richards 1948)) at 25 ºC for three days prior to exposure to substrates. Each soil was loaded into a 48 deep-well plate containing 15 different carbon substrates and one blank (deionized water), in triplicate (Appendix 1). Loaded deep-wells were immediately sealed with a gasket and indicator microplate before incubation for 6 hours at 30 ºC. Indicator stock was made up of Cresol Red, KCl, and $NaHCO_3$, 1:2 agar water to indicator stock was aliquoted into indicator microplate wells.

Substrate-induced microbial catabolic activity was determined via colorimetric detection, as differing amounts of CO_2 respiration led to wells in the indicator microplates expressing color changes (Campbell et al. 2003). Absorbance of each indicator microplate was determined immediately after the 6-hour incubation using a UV-Vis spectrophotometer (Biotek Instruments Inc, Winooski, VT, USA), at 570 nm wavelength. The initial (time zero) post-incubation absorbance values were used to model the amount of CO_2 efflux during respiration using equations provided by MicroResp™, thus allowing for comparisons of the microbial activity at the fire sites.

2.2.6 Statistical Analysis

All statistical analyses were carried out using the vegan package for R software, in addition to the R base program, version 3.5.2. (2018-12-20) “Eggshell Igloo” (Oksanen et al. 2016, R Core Team 2018). Ordinations and figures were produced using ggbiplot and ggplot2 packages for R, in addition to R base plotting functions (Wickham 2009, Vu 2011).

Permutational multivariate analysis of variance (PERMANOVA) was used to test for interaction between environmental data collected at the four burns. The PERMANOVA method is semiparametric in that it allows a classical partitioning of data to be performed, similar to analysis of variance (ANOVA) techniques, while retaining robust statistical properties of rank-based non-parametric multivariate methods (Anderson 2017). PERMANOVA analysis allows data that are non-normal by using distribution-free inferences, with p-values obtained using distribution-free permutation techniques, relying on geometric portioning of multivariate variation with a chosen dissimilarity index (Anderson 2017). Individual BPCA quantities recovered from the fire sites were tested for interaction by PERMANOVA of the Bray-Curtis distance. The same procedure was used to analyze soil microbial respiration rates in response to multiple carbon substrates tested in the community-level physiological profiles.

Non-metric Multidimensional Scaling (NMDS) is a numerical technique that iteratively seeks a solution to a dataset based on a pre-determined number of dimensions with minimal stress (McCune et al. 2002). NMDS ordination is based on a symmetrical matrix of pairwise distances among samples, calculated on a chosen distance measure, in this case the Bray-Curtis distance. A configuration of samples in the chosen number of dimensions is constructed, and the distances among samples in the configuration are regressed against the original matrix. The goodness of fit of the regression is called stress (Holland 2008). The stress value reflects how well the ordination summarizes the observed distances among the samples. NMDS was performed on BPCA quantities and CLPP substrate induced respiration data.

Principal component analysis (Grace-Martin 2013) was performed on heat of combustion, Q (J/g soil), evolution within each temperature region along the differential scanning calorimetry curve. Sample soil organic carbon (g/kg soil), and PyC (g/kg soil) were included in the PCA.

2.3 Results and Discussion

2.3.1 Soil Organic Matter Quality

2.3.1.1 Pyrogenic carbon quantification

When individual BPCA quantities recovered from each site were visualized by NMDS ordination, the results overlapped, indicating no patterns or groupings existed between individual BPCAs or sites (Figure 2.2). Thus, none of the soils was found to have a distinct profile of the six BPCAs recovered (Table 2.1). Site was not found to be a significant grouping factor between BPCA quantities when results were analyzed by perMANOVA (p-value = 0.853).

The sum of individual BPCAs was used to estimate PyC quantity (g/kg soil) (Table 2.1). B horizon PyC quantities were used to scale up these results to estimate PyC at the landscape level at each site. The greatest quantity of PyC was estimated within the 2003 wildfire site, 5167.5 kg/ha. 3685.5 kg/ha PyC was found at the fireguard site, and PyC at the 1968 wildfire and 2008 prescription burn sites was less abundant; 2749.5 kg/ha and 2613 kg/ha, respectively.

PyC found at the 2003 wildfire site is comparable to quantities reported at the site of an intense crown fire in Yosemite National Park, 6400 kg/ha (Tinker and Knight 2000). The level of PyC content estimated in the 2003 wildfire site soil may indicate that, on average, fire intensity was higher at this site than others, however the relatively small area sampled within the burn limits extrapolation of these results. The relationship between fire severity, a product of both fire intensity and duration (Keane et al. 2003), and PyC production is a function of burning conditions and complex intrinsic and extrinsic fuel characteristics, including density, arrangement, moisture and load (Brewer et al. 2013). High intensity fires may produce more PyC as they have higher burning efficiency and affect a greater portion of fuels (Campbell et al.

2007). Considering this, the lower soil PyC content found at the prescription burn site may have resulted from a lower intensity fire with less burning efficiency on average, compared to the recent Vermilion Pass burns (2003 wildfire and fireguard).

Pyrogenic carbon was most abundant in B horizons of all studied soils, (Table 2.1). The areas sampled within the 2003 wildfire and fireguard had the greatest accumulation of PyC in B horizons. Analysis of the profile of BPCAs recovered in A and B horizons separately was completed to identify potential differences in PyC translocation downward through the site soil profiles. While site was not found to be a significant grouping factor in profiles of individual BPCA quantities in separate A or B horizons, (perMANOVA A horizon p-value = 0.819, B horizon = 0.365), NMDS ordination did produce slight differences in distribution. These differences were most noticeable when comparing A and B horizon BPCA profiles from the 2008 Prescription Burn and 1968 Wildfire (Figure 2.2).

The greater abundance of PyC observed in B horizon soils may be a function of long-term accumulation and storage from previous fires. Differences in A and B horizon BPCA profiles may suggest that some forms of PyC, from which certain BPCAs of the six identified here may be more likely to be conjugated through this analysis technique, translocate and accumulate in lower soil horizons faster than others. Previous studies have documented the downward transport of PyC through the soil profile, including both Rodionov et al. (Rodionov et al. 2006) and Hockaday et al. (Hockaday et al. 2006) who showed that transport favors soils with higher water flows, and that fragmentation and dissolution of PyC occurs through oxidation of condensed aromatic ring structure, which favors soluble transport. BPCAs are known to be associated with large aromatic clusters (Schmidt et al. 2001). Further, work in forest soils in northern Michigan concluded that B horizon formation was a function of vegetation and fire frequency, rather than C horizon composition and other soil forming state factors (Mokma and Vance 1989), suggesting that translocation of PyC downward through the soil profile is part of the normal podsolization process. Burn sites in Kootenay National Park could support further study into BPCA molecular marker association with various forms of PyC and how PyC chemical structure influences its rate of translocation through soil.

2.3.1.2 Thermal analysis

Heat of combustion (Q) evolution during differential scanning calorimetry (DSC) was visualized over three temperature regions using boxplots, results were plotted by soil horizon (Figure 2.3 A & B). Heat of combustion is expressed exothermically as Jg^{-1} of soil combusted; thus, more negative values indicate greater energy evolution. The majority of Q evolution from all samples was observed in the lowest temperature region, 150-375ºC. Heat of combustion evolution tended to decrease with increasing DSC temperature. These result suggests that Q evolution was highly related to organic carbon content, rather than PyC content. Prescription burn soils had higher Q evolution than the other fire sites in both upper DSC temperature regions, while both the wildfires and the fireguard soil produced similar Q evolution results in all temperature regions.

Sample mass loss on ignition during thermogravimetric analysis was used to calculate soil organic matter (SOM) and carbon content. Prescription burn A horizons had greater SOM and carbon content that the other fire sites (Table 2.2). The outlying Q evolution results in the lowest DSC temperature region were attributed to these greater SOM and carbon contents, as non-PyC organic carbon likely lacks aromatization structures that increase recalcitrance (Merino et al.

2014). The lowest DSC temperature region is hypothesized to be associated with labile organic carbon structures like carbohydrates and aliphatic compounds (Fernandez et al. 2011).

Principal component analysis of Q evolution and PyC content showed that variation in soil thermal stability was associated with the 375 — 450ºC temperature range in both A and B horizons and accounted for 98 – 95% of data variation in A horizon samples, but only 87% of variation in B horizons. Consistent with organic carbon quantity and heat of combustion at 150 – 375ºC, prescription burn soils displayed an outlying ordination pattern in A horizon samples, but not in B horizons (Figure 2.4).

Prescription burn A horizon samples showed the highest heat of combustion efflux at DSC curve lower temperature regions associated with less recalcitrant compounds, 150-375ºC and 375-450ºC. The difference in thermal stability in prescription burn A horizons is more likely due to their high organic carbon content, rather than PyC content because prescription burn A horizons had the lowest observed PyC in proportion to organic carbon compared to other fire sites.

In the low intensity burning conditions typical of prescribed burning, combustion efficiency is generally low (Certini 2005), so a smaller portion of fuels is exposed to thermal degradation and overall less PyC may be produced (Graca et al. 1999, Schmidt and Noack 2000). As a result, PyC produced by prescribed fires may not be representative in quantity or quality of PyC produced during more intense wildfires (Santin et al. 2016). Johnson and Curtis (2001) observed that after low severity fires it is normal to find increased carbon pool quantities in upper soil horizons because of incorporated unburned or partially burned residues, as well as due transformation of fresh organic matter into more recalcitrant forms. Consistent with their observation, the high quantity of organic carbon found in the prescription burn A horizon studied here may be due to incorporation of partially burned residues into mineral soil.

Previous work by Merino et al. (2014) using both DSC and NMR spectroscopy to reveal changes in soil organic matter after wildfire suggested that monitoring of partial combustion heats and thermal indexes measured by DSC may be useful for estimating SOM quality after fire. This relationship between heat of combustion efflux and organic carbon is further supported by the thermal stability profiles of B horizons across fire sites presented in this study. B horizon thermal stability profiles were not distinct from each other (Figure 2.4), consistent the similar quantities of PyC found in the studied soils. These results suggest that SOM stability following prescription burning is in part dependent on fire severity, and that at lower intensities prescription burning is not able to consume or thermally alter SOM enough to increase its recalcitrance and therefore, stability, to the extent of wildfire.

2.3.2 Community-level Physiological Profile

The substrate induced respiration (SIR) responses of the microbial community at the prescription burn and 2003 wildfire were separated from the wildfire sites in ordination space from the 2003 fireguard and 1968 wildfire soil communities, which display a high degree of overlap (Figure 2.5). The combined SIR responses from each site form a profile of the microbial community physiology. Significant grouping by site was found by perMANOVA (p-value = 0.011, $R^2 = 0.81$).

In combination with the high SOM but low quantity of A horizon PyC, the microbial community physiological profile suggests that the prescription burn behavior and severity could be having long-term influence in steering the soil microbial community. After fire, the altered soil environment may cause some microbial groups to become dominant, leading to changes in community composition and structure. Thermally altered soil resources like available organic carbon, nutrients and water, as well as abiotic soil characteristics like pH influence microbial community growth and composition (Lehmann et al. 2011). Heavy coarse woody debris surface fuel buildup at the prescription burn site could have contributed to lower oxygen availability during burning resulting in incomplete smoldering combustion, leaving behind less PyC or partially thermally degraded organic residues on the soil surface (Gray and Kubian 2010). Both climatic factors that limit decomposition, and ground fuel load influence the depth of burn and contribute to higher soil organic matter (SOM) persistent on-site years after burning (Johnson and Curtis 2001). Thermal analysis of the PB site A horizon soil revealed that its organic C content was highly labile. The microbial community at this site may have adapted its physiology to use this resource, explaining its distinct community-level physiological profile. These soil properties may be early indicators that this site will recover to support a different vegetation community compared to the wildfire sites (Hart et al. 2005). This site could be an ideal location for repeated application of PB to support restoration of soil properties from the effects of fire exclusion, and to determine if repeated application of low severity fire can lead to soil conditions similar to those following wildfires over the long term.

2.3.3 Study Limitations

The objective of this study was to use soil analysis techniques to provide insight on the effectiveness of prescribed burning at mimicking the soil conditions created by wildfire in Kootenay National Park (KNP). Unfortunately, this study’s survey design limits the scope of inference that can be drawn from the data collected. Burned landscapes are spatially heterogeneous because fire behavior is not uniform within a forest. Large-scale and thoughtful sampling design is required to collect data that accurately represent this spatial diversity. Studies such as this, where samples were taken in a relatively small area within a burned landscape, therefore, have limited range for data interpretation. The sampling in this study was not designed to effectively capture the spatial heterogeneity within the burn sites. Three 100 m transects spaced 10 m apart represent approximately 2000 m^2 sampling area within burn sites that are thousands of hectares.

A further challenge in studying soil properties following fire is the difficulty finding replicate sites when studying naturally occurring fires, (as opposed to experimental burn plots). These four studied sites could not be considered replicates due to high degrees of variation in site ecology, recovery time, and fire behavior. Fire ‘type’ based on ignition cannot be used as a factor in statistical analysis and inferences made from data collected at these sites must acknowledge the limitations of this study.

Study of the impacts of burning using soil analysis techniques is limited by their reliance on PyC production and deposition into soil. Interpretation of results is made more complex because PyC production does not have a linear relationship with fuels present in the burn area, rather it is a factor of both fuel load and fire behavior, which is influenced by numerous natural conditions (Brewer et al. 2013, Santin et al. 2016).

2.5 Conclusions

This study characterized post-burn soil conditions within four fire sites in Kootenay National Park. The objective of this study was to use soil analysis techniques to determine if prescribed burning lead to similar soil conditions as those created by wildfires, however due to sampling design limitations, the scope of interpretation was constrained to within burn trends and site comparison, rather than analysis for significant difference between sites. Pyrogenic carbon quantity (PyC), soil organic matter (SOM) quantity and thermal stability, and microbial community physiology were analyzed to gain insight into the ecology of the studied forests as they recover from fire.

PyC was most abundant within the 2003 wildfire area in Vermilion Pass. The quantity of PyC found here was similar to published quantities resulting from a high intensity wildfire in a similar forest type (Tinker and Knight 2000). The lower PyC content, higher SOM content leading to lower thermal stability, and distinct microbial community physiological profile, at the prescribed burn site suggest that area burned with lower severity than was reached at the wildfire sites. Lower fire severity is typical of prescribed burns, where fuel management goals must be balanced against moister and cooler environmental conditions that allow a greater chance of control of the burn but are not typical of environmental conditions during wildfires (Keane et al. 2003, Ryan et al. 2013). Due to the strong interrelationship between soil microbes and vegetation (Hart et al. 2005), low severity prescription burning in forests with heavy fuel loads may create soil properties with lasting, deterministic effects on future forest species structure. Greater PyC content was found in all fire site B horizons compared to A horizons, and within B horizons, the greatest quantity of PyC was found at the 2003 wildfire site. This result may be indicative of accumulation of PyC through translocation to lower soil horizons within the 10 years from the burn to when samples were taken.

This study showed that soil analysis techniques have the potential to be an effective tool to provide insight on the effectiveness of using prescribed burning (PB) as a surrogate for wildfire in restoring and maintaining soil health after periods of fire exclusion, however careful study design must be implemented to collect data that supports interpretation of land-scape level effects. Soil analysis using the PyC quantification and thermal stability characterization techniques, as presented here, could be expanded in Kootenay National Park to allow deeper understanding of long-term trends in soil development after fire. Studies should include the degree to which soil properties altered by fire, including PyC and organic C content, fertility, and microbial community physiology, shape recovering vegetation communities over time. Prescribed burning programs that cannot burn at severities typical of regional wildfire regimes may not adequately restore soil properties to pre-fire exclusion conditions, causing unintended and lasting impacts on forest composition.

Table 2.1. Kootenay National Park soil benzene polycarboxylic acid recovery results for pyrogenic carbon quantification. High pressure liquid chromatography (HPLC) was used to quantify BPCA molecular markers of PyC. Site codes are wildfire – WF, fireguard – FG, prescribed burn – PB.

		BPCA (μg)						Sum BPCA (g kg^{-1} Soil ± SE)	Pyrogenic Carbon (g kg^{-1} Soil ± SE)
Site	Horizon	B6CA	B5CA	1,2,4,5-B4CA	1,3,5-B3CA	1,2,4-B3CA	1,2,3-B3CA		
1968 WF	A	61.968	13.350	20.787	24.492	31.499	0.000	1.10 ±0.47	2.49 ±1.07
1968 WF	B	96.209	30.145	25.282	29.591	20.490	0.710	1.41 ±0.23	2.59 ±0.52
2003 WF	A	57.336	16.744	20.496	26.672	14.250	1.240	0.99 ±0.19	2.24 ±0.43
2003 WF	B	179.810	79.776	34.787	31.742	24.386	0.000	2.65 ±0.19	6.02 ±0.43
2003 FG	A	63.715	35.205	24.777	26.327	17.477	0.000	1.16 ±0.37	2.63 ±0.84
2003 FG	B	149.975	46.413	26.606	41.235	16.557	0.000	1.89 ±0.44	4.29 ±1.00
2008 PB	A	98.388	6.168	23.288	28.374	28.117	7.914	1.51 ±0.21	3.43 ±0.48
2008 PB	B	111.350	39.451	25.242	25.350	7.743	0.000	1.34 ±0.15	3.04 ±0.34

Table 2.2. Fire site soil proportions of organic matter, organic carbon, and pyrogenic carbon. Loss on ignition data generated during thermogravimetric analysis were used to determine the total organic matter content in each sample, n = 3. Total organic carbon was estimated as 0.588 of sample organic matter. Pyrogenic carbon was estimated in proportion to sample organic carbon based on cumulative BPCAs recovered by HPLC.

Site	Horizon	Organic Matter (% sample weight ± SE	Organic Carbon (% sample weight ± SE.)	Pyrogenic Carbon (% Org C ± SE)
1968 WF	A	5.32 ±1.00	3.13 ±0.21	9.94 ±2.12
1968 WF	B	5.64 ±0.22	3.31 ±0.05	9.56 ±0.46
2003 WF	A	5.90 ±0.93	3.47 ±0.19	6.88 ±0.69
2003 WF	B	8.62 ±0.60	5.07 ±0.12	12.00 ±0.50
2003 FG	A	5.03 ±1.18	2.96 ±0.25	10.03 ±1.60
2003 FG	B	5.21 ±0.63	3.06 ±0.13	14.12 ±0.98
2008 PB	A	33.58 ±5.83	19.75 ±1.21	1.77 ±0.06
2008 PB	B	3.35 ±0.60	1.97 ±0.12	15.90 ±0.41

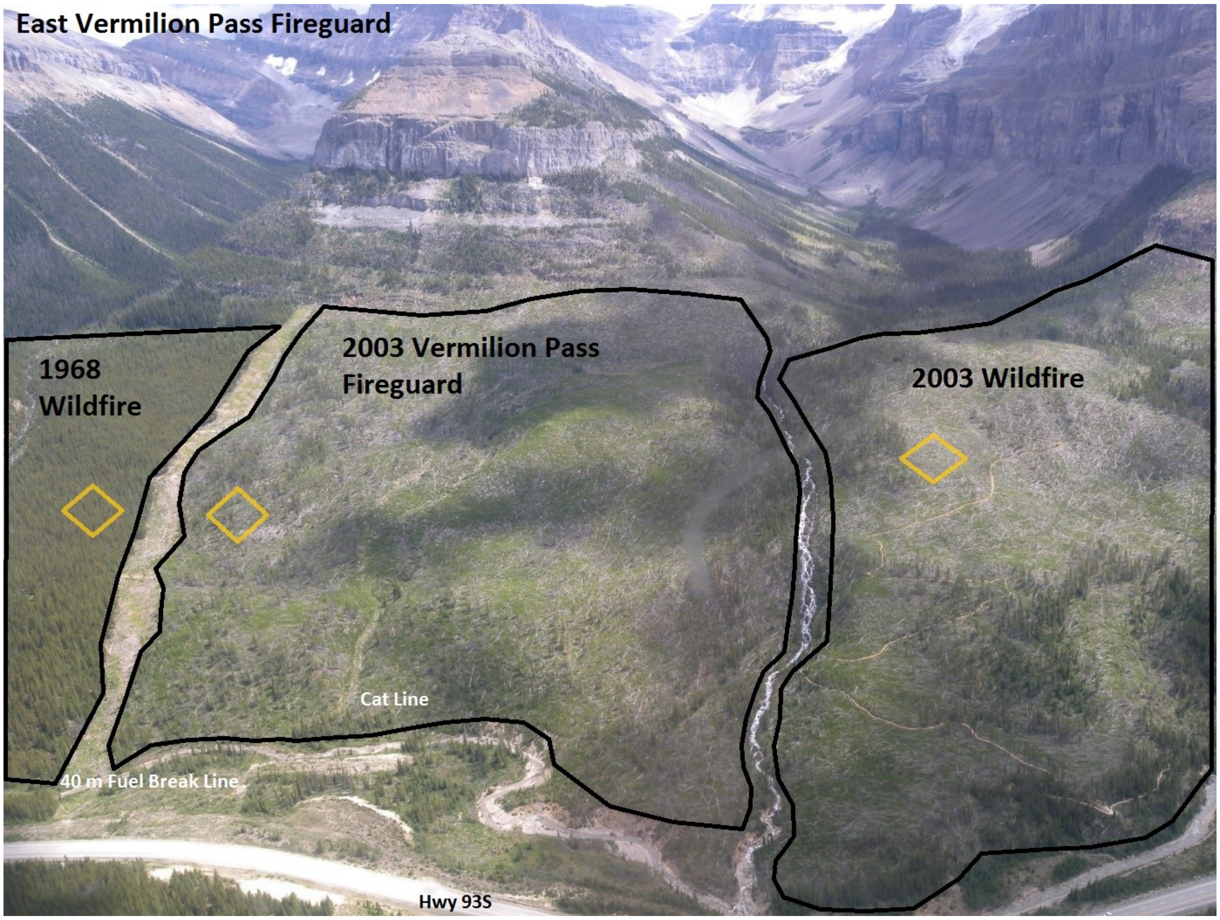


Figure 2.1. Aerial view of Vermilion Pass East fireguard in Kootenay National Park, Canada. From left to right, the forest burned in 1968 ('1968 Wildfire') and 2003 ('2003 Wildfire'), with its continuity broken by the 2003 Fireguard ('2003 Fireguard'). Yellow squares indicate areas where transect lines were located for soil sampling. Cat lines are visible to the right of the fireguard. The fourth site sampled, '2008 Prescribed Burn' is located 67 km from Vermillion Pass and is not pictured. Transect overlay not to scale; for illustrative purposes only. Photo Credit Charlie McLellan, Parks Canada (2016).

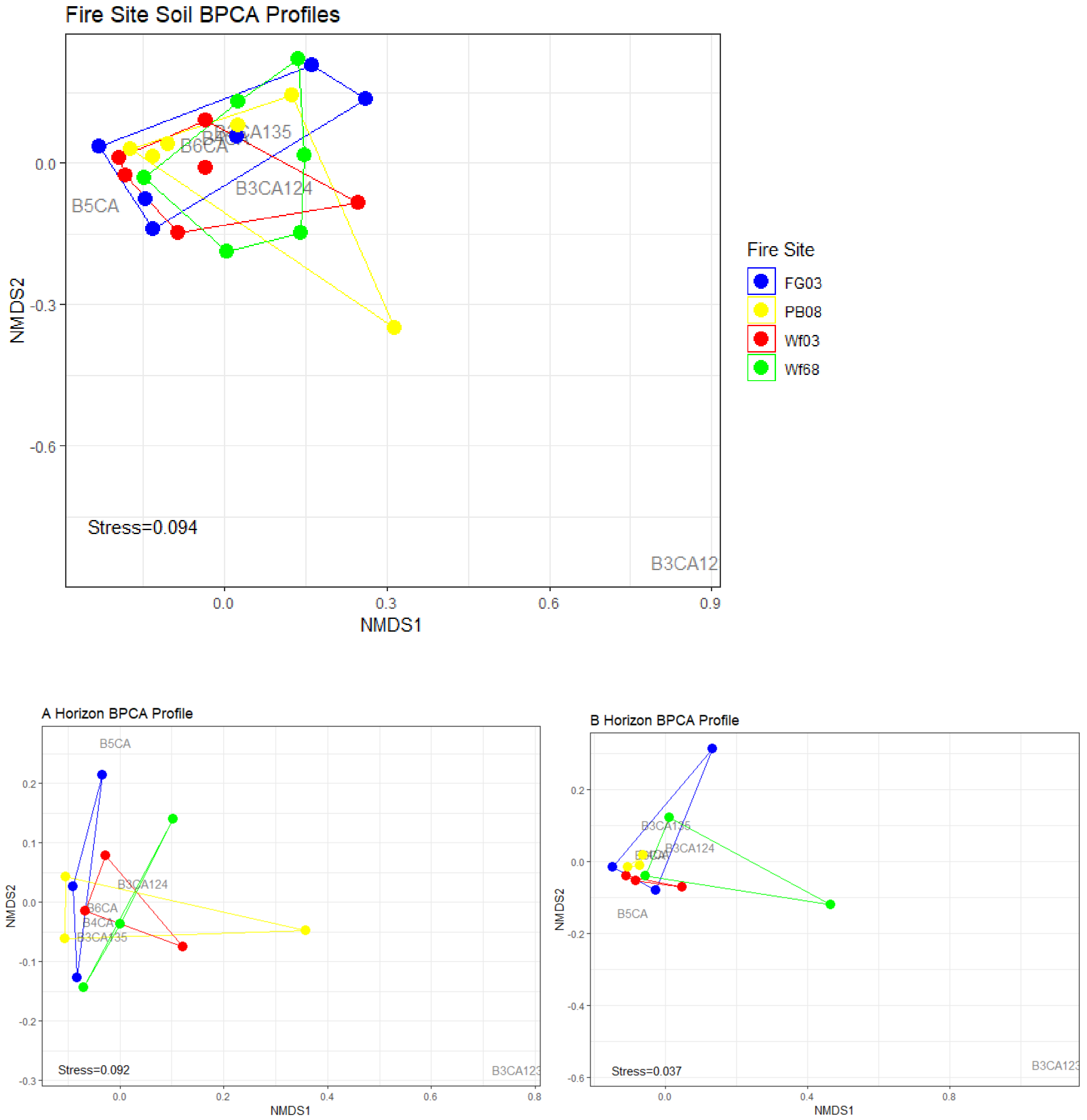
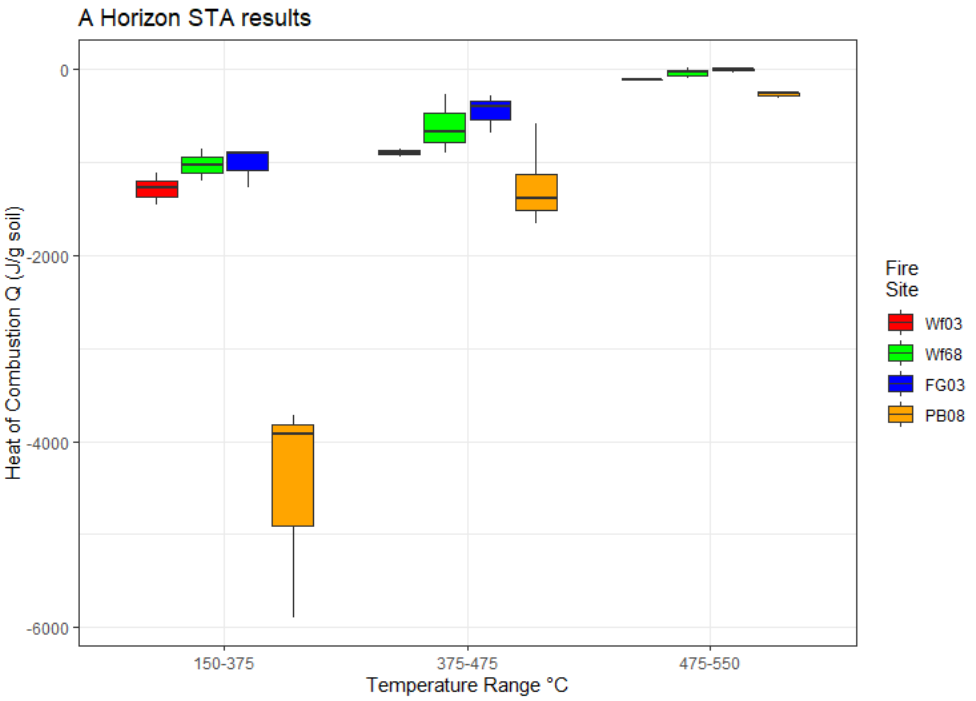
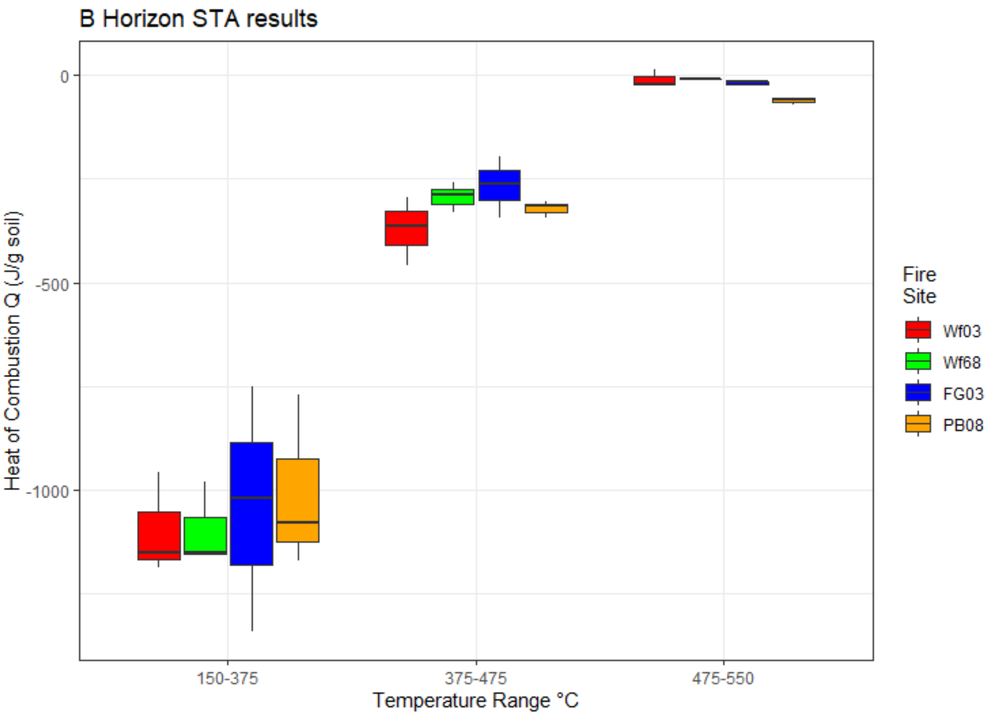


Figure 2.2. BPCA profiles in Kootenay National Park fire site soils. NMDS was calculated using Bray-Curtis dissimilarity. perMANOVA shows that there is no significant grouping difference among sites (p-value = 0.858). BPCA A horizon data (p-value = 0.804), BPCA B horizon data (p-value = 0.352). Figure legend "FG03" refers to the fireguard site, "PB08" refers to the

prescribed burn site, “WF03” refers to the 2003 wildfire site, and “WF68” refers to the 1968 wildfire site.


A Horizon STA results
Heat of Combustion Q (J/g soil)
0
-2000
-4000
-6000
150-375
375-475
475-550
Temperature Range °C
Fire Site
Wf03
Wf68
FG03
PB08

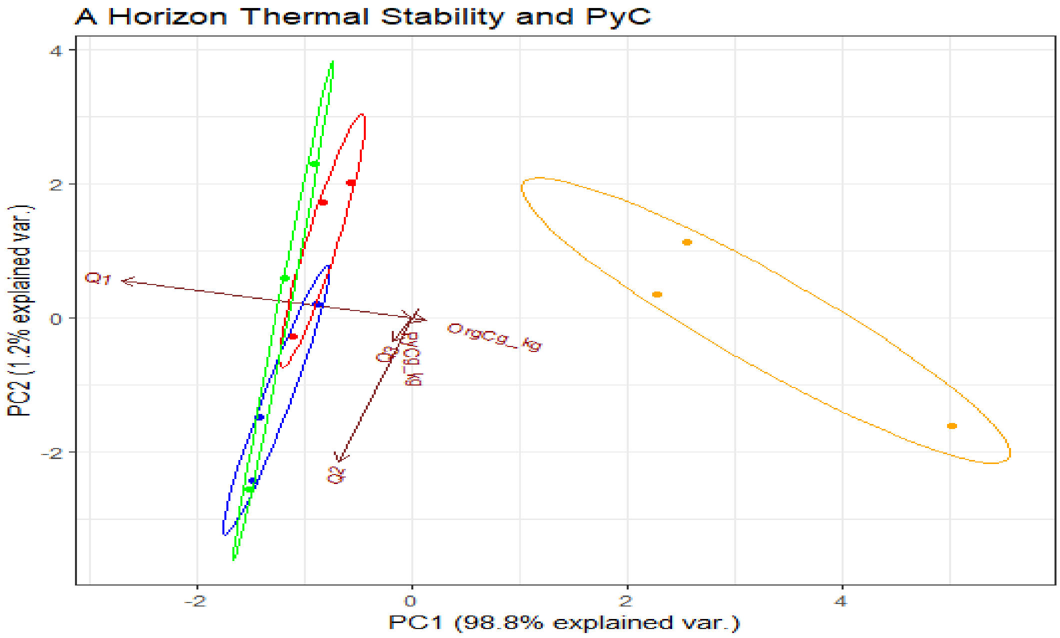
A)


B Horizon STA results
Heat of Combustion Q (J/g soil)
0
-500
-1000
150-375
375-475
475-550
Temperature Range °C
Fire Site
Wf03
Wf68
FG03
PB08

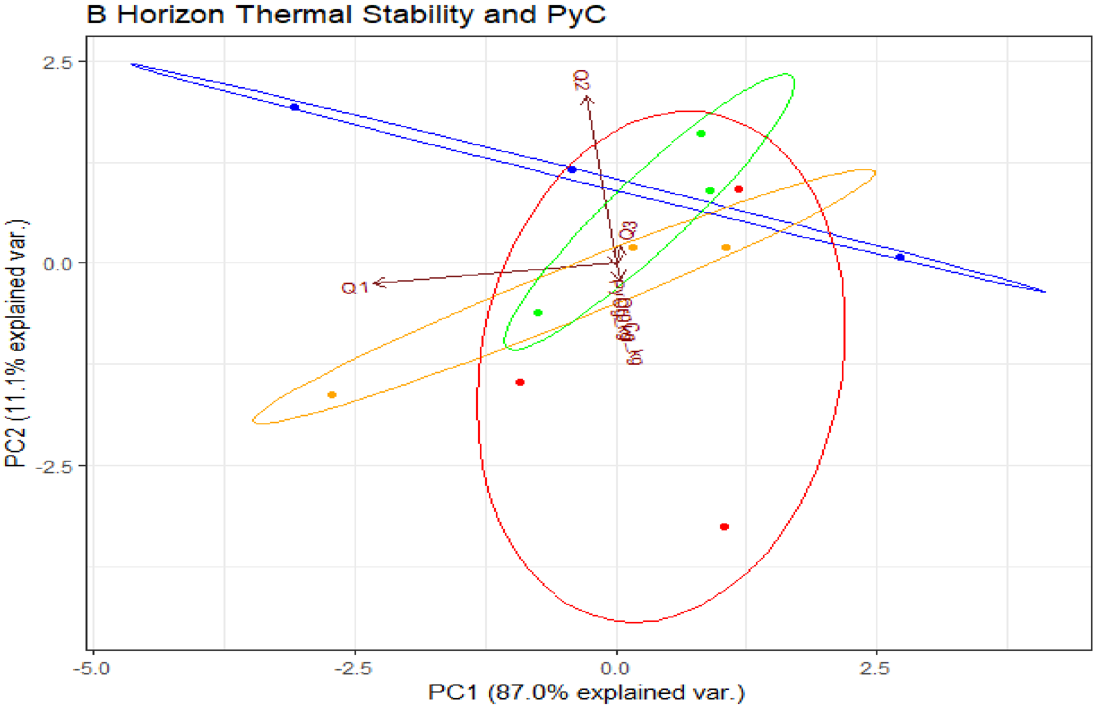
B)

Figure 2.3. Organic matter stability in Kootenay National Park burn site soils. Differential Scanning Calorimetry (DSC) shows the heat of combustion (Q), evolved from Kootenay National Park fire site soil samples in three temperature ranges. Q is expressed exothermically as J/g of soil, thus more negative values indicate greater energy evolution. Figure a) prescribed burn A horizon soils show outlying large Q evolution in the lowest DSC temperature region, b) removal of the outlying PB08 A horizon results allows visualization of similar Q evolution across sites at the higher temperature regions. Figure legend “FG03” refers to the fireguard site, “PB08” refers to the prescribed burn site, “WF03” refers to the 2003 wildfire site, and “WF68” refers to the 1968 wildfire site.

A)


A Horizon Thermal Stability and PyC
4
2
0
-2
PC2 (1.2% explained var.)
Q1
OrgCg_kg
Q3
PyCg_kg
Q2
-2
0
2
4
PC1 (98.8% explained var.)

B)


B Horizon Thermal Stability and PyC
2.5
0.0
-2.5
PC2 (11.1% explained var.)
Q2
Q3
Q1
PyCg_kg
OrgCg_kg
-5.0
-2.5
0.0
2.5
PC1 (87.0% explained var.)

C)

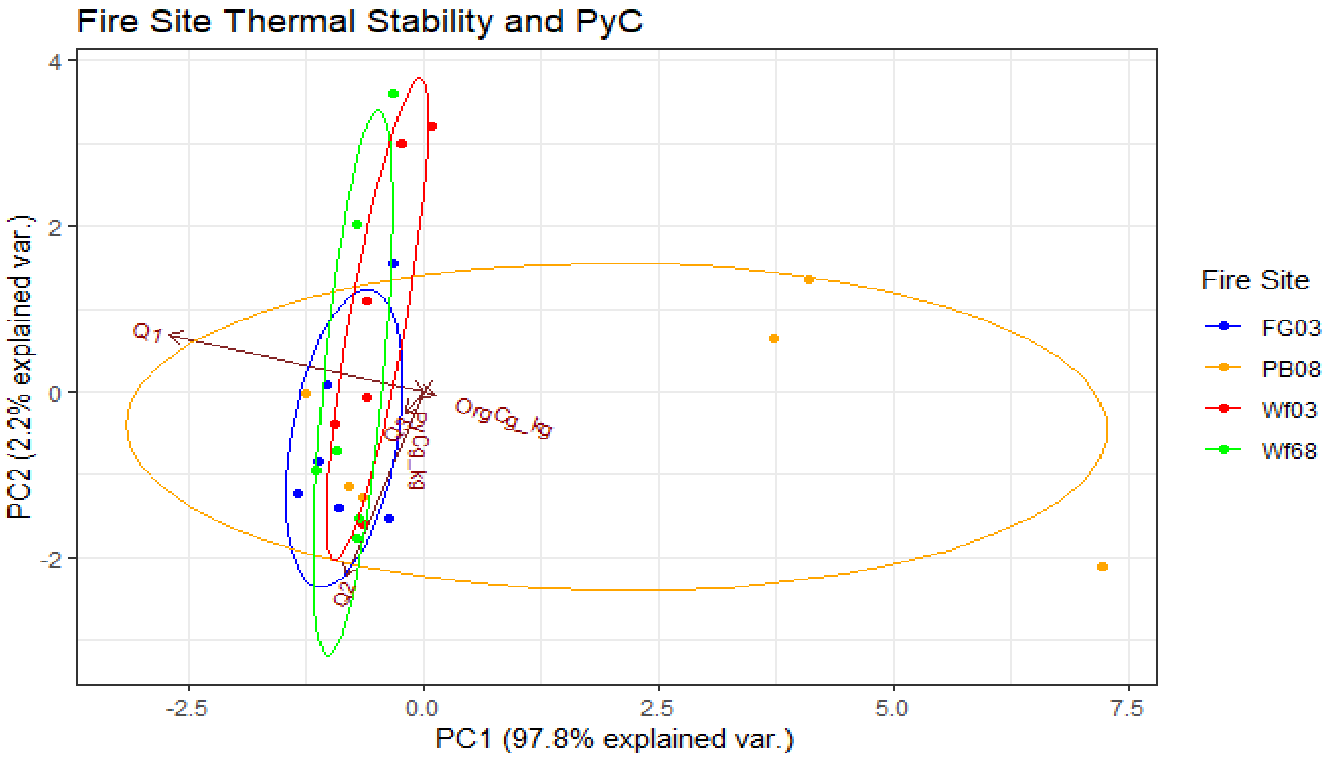


Figure 2.4. Fire site thermal stability, pyrogenic carbon and organic matter quality. Biplot visualization shows the results of principal component analysis of fire site soil thermal stability and PyC quantity. Factors included in the analysis were heat of combustion, Q (J/g), evolved across three DSC temperature ranges, Q1; 150-375°C, Q2; 375-475°C, and Q3; 475-550°C, and quantity of soil organic carbon g/kg, and pyrogenic carbon g/kg soil. Panel A shows results of A horizon analysis, panel B shows results of B horizon analysis, and panel C shows results of total soil sample analysis. Figure legend “FG03” refers to the fireguard site, “PB08” refers to the prescribed burn site, “WF03” refers to the 2003 wildfire site, and “WF68” refers to the 1968 wildfire site.

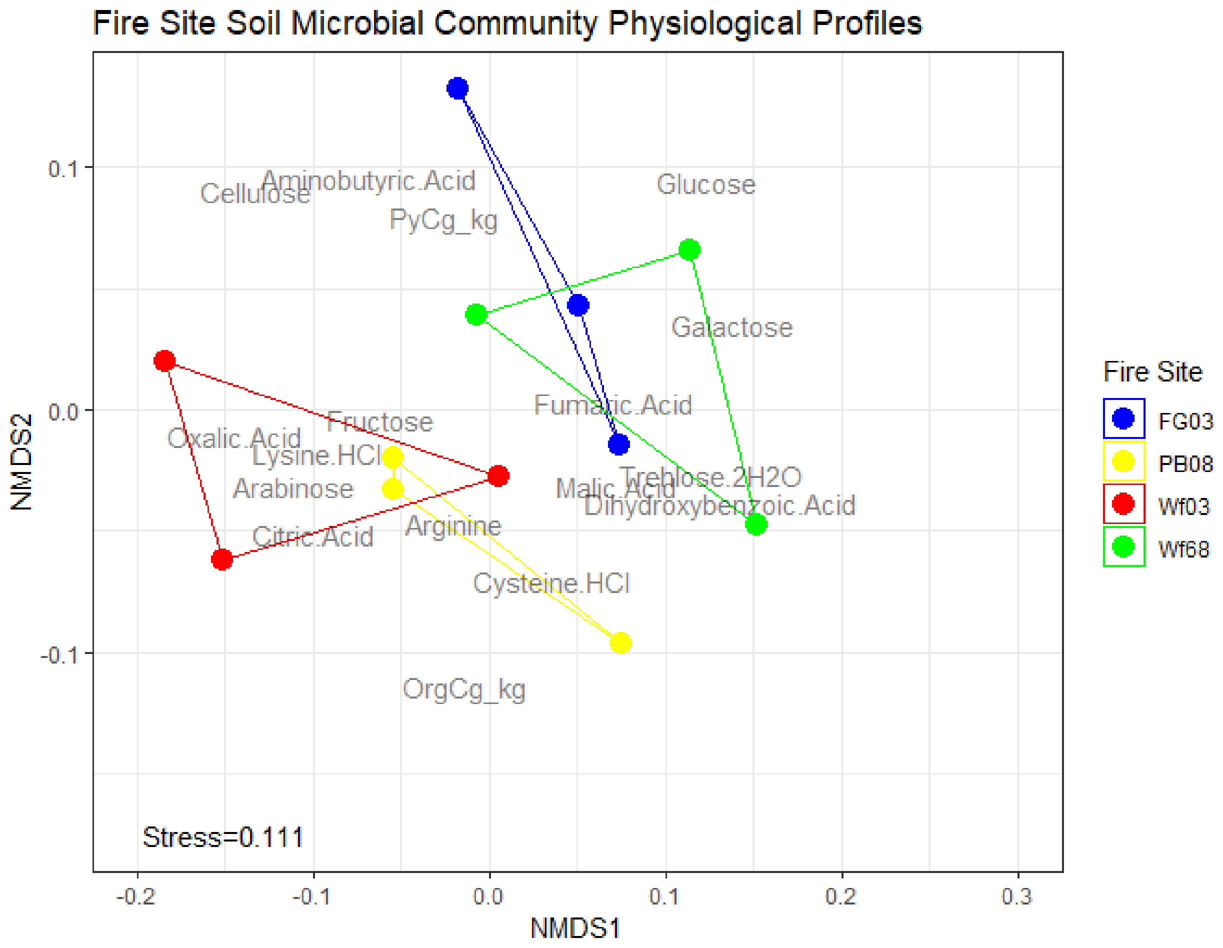


Figure 2.5. Community-level physiological profiles in fire site soils. The relationship between microbial CO_2 respiration responses to single carbon substrates, and soil organic carbon and PyC contents were tested for statistical significance and visualized using ordination. NMDS was calculated using the Bray-Curtis dissimilarity, perMANOVA results support significant separation among the fire study sites (p-value = 0.011). Figure legend “FG03” refers to the fireguard site, “PB08” refers to the prescribed burn site, “WF03” refers to the 2003 wildfire site, and “WF68” refers to the 1968 wildfire site.

Chapter 3: Biochar amendment has positive effects on growth in *Pinus banksiana* ectomycorrhizal symbioses

3.1 Introduction

Ectomycorrhizal (ECM) fungi typically form symbioses with woody plants (Smith and Read 2008). Many tree families (Pinaceae, Betulaceae, Salicaceae) form mutualistic symbioses with a diverse array of soil fungi that obtain and transfer soil nutrients from mineral and organic sources to their plant hosts in exchange for photosynthetically derived carbohydrates (Smith and Read 2008). A growing body of research demonstrates that mycorrhizal fungi have large effects on plant growth (van der Heijden 2002). Work in northeastern Alberta has shown that Jack pine form symbioses with at least 56 species of mycorrhizal fungi (Danielson 1984, Danielson and Visser 1989). Several ectomycorrhizal fungal families, including *Suillus*, are specific to pines and do not form mycorrhizae with other conifers (Bruns et al. 2002). *Suillus* inoculation has been shown to enhance pine seedling survival under harsh, competitive conditions like those created after fire (Lonergan et al. 2014).

Wildfire is a natural disturbance and essential ecosystem process in Rocky Mountain and boreal forests. In these regions, fire exclusion over the past century has altered stand structures to increase flammability and fuel loadings, as well as allowed thick duff, litter, and organic material horizons to accumulate on soils (Keane et al. 2003). Combustion of thick organic horizons results in deep soil heating that can cause high microorganism and plant propagule mortality (Hungerford et al. 1991). Mycorrhizal fungi colonize organic and upper mineral soil horizons and are therefore sensitive to deep soil heating that results from burning thick organic material accumulations (Borchers and Perry 1990). High severity fires caused by abnormally high surface fuel loads after fire exclusion can severely reduce mycorrhizal populations and create marked effects on ECM community composition (Debano 1991, Visser 1995). In addition to the lethal direct effects of soil heating, fire indirectly affects the persistence of mycorrhizal fungi by removal of their symbiotic host plants which tends to decrease mycorrhizal survival (Amaranthus and Perry 1987, Allen 1991). In contrast, low intensity fires where part of the organic layer remains unburnt and some trees survive, ECM fungi may have a role in improving plant health and survival in recovering forests (Jonsson et al. 1999).

Charcoal is a ubiquitous and persistent component of soils in fire-disturbed ecosystems. Evidence of paleo-wildfires in the Famennian age, 372.2 – 358.9 million years ago, (Devonian/Carboniferous periods), (Scott 2000, Prestianni et al. 2010) suggest that wildfire is concomitant with the origin of terrestrial plants and has played an important role throughout the history of life (Pausas and Keeley 2009). The pyrogenic carbon (PyC) compounds left behind in soil after fire alter the physicochemical and biological environment and persist in soils for centuries due to their polycyclic aromatic carbon structure (Glaser et al. 2002, Hart et al. 2005).

In an effort to restore fire to contemporary forests, land managers are using both prescribed burning and wildfire management; allowing fires in remote areas to burn without aggressive suppression (Weber and Taylor 1992, Taylor 1998). In boreal soils, typically 0.7 – 2.0% of organic matter is converted to PyC during a wildfire, however, variation in fuel conversion to PyC is influenced by factors that differ between wildfires and prescribed fires; including fire temperature, residence time, fuel type and fuel size (Demirbas 2004, Czimczik et al. 2005, Demirbas 2006). Fire exclusion in ecosystems where historically fire was a dominant natural disturbance has allowed woody fuel accumulation and changes in stand canopy structure that increase the likelihood of high severity wildfires when the forest does burn (Keane et al. 2003). High severity wildfires have the potential to contribute more charcoal to soils, for example 6400 kg/ha (0.64 kg m²), and 5168 kg/ha (0.52 kg m²), of charcoal was found after intense crown fire in conifer dominated forests in Yosemite National Park, and Kootenay National Park (Tinker and Knight 2000). Burning conditions and ignition patterns that allow anthropogenic control of prescribed fires can contribute to lower burn severities and charcoal deposition (Knapp et al. 2009). Prescribed burning in conifer forests has been found to deposit a range of charcoal quantities depending on fuel conditions, from 2613 kg/ha (0.26 kg m²), under heavy fuel load in Kootenay National Park, to less than 500 kg/ha in a northern Jack Pine forest (Lynch et al. 2004).

The impact of varying levels of charcoal left behind by wildfires in soil on boreal ECM fungal communities remains largely unknown, however charcoal amendment was shown to have positive effects on pine shoot growth and tended towards higher numbers of ECM colonized root tips (Robertson et al. 2012). Four potential mechanisms by which charcoal could influence mycorrhizal abundance and functioning have been suggested; (a) altered nutrient availability and physico-chemical properties, (b) indirect effects on ectomycorrhizas through effects on other soil microbes, (c) plant-fungi signaling interference and detoxification of allelochemicals, and (d) provision of a refugia from fungal grazers (Warnock et al. 2007). During charcoal formation, oxidation produces carboxylic acids on the edges of charcoal's polycyclic aromatic backbone structure. These carboxylic acids increase charcoal's nutrient and water holding capacity and may be responsible for charcoal's role in altering nutrient availability and the soil physicochemical environment (Glaser et al. 2002). The effects of charcoal on plant-fungal signaling may arise because charcoal can sorb diverse organic molecules, including small non-polar molecules used by soil microorganisms for intercellular communication (Smernik 2009, Lehmann et al. 2011, LeCroy et al. 2013).

The objective of this study was to investigate how differing quantities of charcoal affect the formation of ectomycorrhizal symbioses and growth of ectomycorrhizal pine. To meet this objective ectomycorrhizal *S. tomentosus*–jack pine seedlings were grown in microcosms with three levels of biochar amendment, reflective of a high severity and low severity burn given published values. Pine seedling radicle length and fungal mycelial area were measured to capture the impact of biochar amendment on growth. Ectomycorrhizal symbiosis was confirmed by microscopic visualization of 'Hartig Net' structures in stained root tip cross-sections. By working in microcosms, I isolated the pine-fungal ectomycorrhizal symbiosis from other soil microbes and fungal grazers.

3.2 Methods

3.2.1 Experimental Design—Overview

Microcosms were constructed to test the effect of biochar on growth of ectomycorrhizal symbiotic partners *Pinus banksiana* and *Suillus tomentosus* individually, and together, in a factorial study with three levels of biochar application. The effect of biochar application was assessed on fungal mycelial area and pine radicle length. Ectomycorrhizal symbiosis was confirmed via the presence of a 'Hartig Net' on the pine radicle tip by cross-sectional tissue sampling and staining for confocal microscopy. Non-mycorrhizal microcosms containing only *S. tomentosus* or *P. banksiana*, and mycorrhizal microcosms containing both species were constructed at three biochar amendment levels; no biochar, low biochar, and high biochar (Figure 3.1). Forty (40) of each non-mycorrhizal and mycorrhizal microcosm were constructed at each biochar treatment level (360 total microcosms). The microcosm methodology used in this study was adapted from Jones *et al*. 2013.

3.2.2 Biochar Synthesis

To meet experimental demands for PyC, biochar was used in this study as a surrogate for field collected charcoal. Biochar is a synthesized form of PyC that falls within the black carbon continuum and shares the characteristics of that continuum; including aromatization, high surface area, and recalcitrance to degradation (Schmidt and Noack 2000, Preston and Schmidt 2006). Biochar is typically synthesized from organic materials by slow pyrolysis, under oxygen-limited conditions, in a range of temperatures between ~ 400 – 500ºC (Lehmann et al. 2006). I applied biochar at two levels, low and high, representative of published quantities of charcoal found within the top 15 cm of soil on sites of coniferous forest wildfires (Ohlson et al. 2009). The high biochar application rate, 0.00416 kg/L is representative of 0.624 kg m^2, consistent with charcoal quantity found in coniferous forests after intense crown fire activity (Tinker and Knight 2000). The low biochar application rate, 0.00208 kg/L or 0.312 kg m^2, representative of moderate fire intensity given it is half the quantity of charcoal deposited after an intense crown fire. For purposes of experimental design and the above calculations, the quantity of charcoal was assumed to be uniform by depth.

Biochar was prepared from Jack Pine (*Pinus banksiana)* and Scots Pine (*Pinus sylvestris L.)*, branches and needles collected from the North Saskatchewan River valley near Fort Saskatchewan, Alberta. Branches were trimmed to approximate 3 cm pieces using pruning shears in the laboratory. The two species were mixed after trimming to a 1:1 mixture ratio. 100 g of mixed material was wrapped in foil, placed in a foil dish and embedded in sand to create an anaerobic environment. The dish was placed in a cold muffle furnace ramped over 30 mins to 500 ºC, then held for 2 hours. After heating, the foil dish was cooled on benchtop approximately

1 hour prior to opening. Charred material was weighed, generally 100 g of fresh pine yielded ~ 40 g of biochar. The biochar produced had a carbon content of 76.56 % (w/w).

The charred pine was ground using a mortar and pestle to a uniform, fine texture. After grinding, the biochar was washed with deionized water to remove ash material. Washing consisted of placing 200 g of biochar in 700 ml of deionized water in a 1000 ml plastic Erlenmeyer flask on a shaker table for 30 minutes. After washing, biochar filtered out of solution using vacuum filtration and allowed to dry.

Biochar surface area was analyzed with BET theory and found to be 1100 m^2 /kg. A sample of biochar was heated to 105 °C for 24 hours prior to being analyzed for surface area. The surface area of biochar used in the microcosms was analyzed using Quantachrome Autosorb iQ, samples were outgassed at 105 ºC for 8 hours. Samples were characterized by the University of Alberta Nanofabrication and Characterization Facility.

3.2.3 Source of biological materials

A live culture of *Suillus tomentosus* was obtained from the private collection of Dr. Jonathan Cale, University of Alberta. *Suillus tomentosus* was maintained on Modified Melin-Norkrans (MMN) agar in 110 mm dishes. A 5 mm core was taken from the existing culture using a flame sterilized cork borer every 30 days and placed on fresh MMN agar in a 110 mm dish and sealed with a double layer of Parafilm™ to maintain the culture.

Pinus banksiana seeds were purchased from Tree Time in Edmonton. Seeds were collected from Alberta Seed Zone CM3.1. Seeds were soaked in cold tap water for 48 hours, then placed in a 2 °C refrigerator for 14 days on a moist paper towel. Paper was misted with water to maintain seed moisture throughout this time. Prior to surface sterilization, seeds were soaked for

24 hours in cold tap water. The seed preparation methodology used in this study was adapted from Hankin *et al*. 2015.

Seeds were surface sterilized by 5 min exposure to 30% w/w hydrogen peroxide. Groups of 10 seeds were placed in 50 ml of 30% hydrogen peroxide in a 125 ml Erlenmeyer flask and bubbled for 5 minutes. A bubbler device was constructed using 10 mm tubing and a 1 ml pipette tip. The top of the Erlenmeyer was covered with foil, accommodating the bubbler tubing. At 5 minutes 100 ml of sterile cold water was added to the Erlenmeyer flask under sterile lab conditions maintained by a Bunsen burner. Seeds were extracted from the liquid using flame sterilized tweezers immediately and placed onto prepared microcosm agar dishes. See Appendix 2 for seed surface sterilization method development.

3.2.5 Microcosm assembly

Microcosm were prepared using sterile technique; all work was carried out in a Biological Safety Cabinet (BM4-2A-49, Canadian Cabinets Company Ltd., Ottawa) outfitted with an intermittent Bunsen burner for flame sterilization of metal equipment.

All microcosms contained MMN growth media agar gel. MMN media was prepared as per manufacturer instructions with additional agar (bioPLUS Melin-Norkrans Medium, Modified without agar, Appendix 2). Media mixture was heated to 100 °C, stirring continuously. After cooling on benchtop for approximately 10 mins, 25 µg/mL of filter sterilized streptomycin was added to inhibit bacterial growth. pH was controlled to 5.6-5.8 using 1 Molar HCl. After cooling for an additional 10 minutes, media was poured into labelled 110 mm Petri dishes to a depth of 1 cm, allowed to cool a further 10 minutes to solidify, then covered with supplied covers and sealed with Parafilm™. Petri dishes containing MMN were stored in a 4 ºC refrigerator if not used immediately.

For microcosms including a host, one surface sterilized jack pine seed was placed, point towards the middle of the dish, on prepared MMN Petri-dish using a flame-sterilized inoculation loop. The dish was sealed with two layers of Parafilm™ and wrapped in foil. For microcosms including the fungus, cores for inoculation of microcosm plates were taken from the mycelium edge of an established *S. tomentosus* culture using a 0.14 cm^2 cork borer. The *S. tomentosus* core was placed 1 cm from the edge of prepared MMN Petri-dish. The dish was sealed with Parafilm™ and wrapped in foil. All microcosms were stored vertically, fungal core at bottom, seed at the top, in a growth cabinet.

Microcosms containing pine seedlings with radicles 5 cm or longer after 30 days in the growth cabinet were notched and the needles of the pine seedling gently extracted. Microcosms were notched using a flame-heated metal spatula to melt a small hole (less than 1 cm wide/tall) into the lid and edge of the Petri-plate. Pine seedlings were wrapped and notches were sealed with Millipore™ tape. After notching and taping, Parafilm™ was replaced and the microcosm was wrapped in foil again, excluding the pine needles. Microcosms were removed from the experiment if they showed visible contamination, or for microcosms containing pine, if the radicle failed to emerge from the seed by day 30 in the trial.

Microcosms were stored in a growth cabinet in the Agriculture and Forestry building, University of Alberta. Microcosms were kept vertical in specifically made racks constructed from PVC pipe lengths with cross sectional cuts sized to accommodate 110 mm microcosm dishes. Growth cabinet temperature was maintained at 21 ºC, with a 12 hour photo-period. Relative humidity in the growth cabinet was maintained at 65%.

3.2.7 Photography and Image Measurements

Microcosms were photographed at 7 day intervals for 60 days using a Pentax Optio WG-2 GPS digital camera. Measurements of seedling radicle length and fungal surface area were taken using ImageJ 1.x software (Schneider et al. 2012).

Radicle tip and fungal mycelium samples were taken using a 0.50 cm cork borer at day 30 and 60 in the experiment. After sampling, root tips and fungal tissue was stored for one week in Formaldehyde-Formalin-Acetic Acid, (FFA), a tissue fixative supplied by the Biological Sciences Microscopy Unit. Samples were embedded in wax blocks. Eight µm cross-sections of pine radicle tip and fungal mycelium were cut using a microtome, then stained with Toluidine Blue. Toluidine Blue is a basic thiazine metachromatic dye that displays high affinity for acidic tissue components like DNA and RNA. It specifically binds to carboxylic acids, sulfates, phosphate radicals, and nucleic acids. Tissues stained with Toluidine Blue appear light to deep royal blue (Epstein et al. 1992, Epstein et al. 1997).

Prepared slides were examined under 10x and 40x power for structures indicative of ectomycorrhizal symbiosis, namely, the ‘Hartig Net’, fungal mantle, and intercellular hyphal clamps. Images were taken via Olympus Confocal microscope and image capture software.

3.2.8 Statistical Analysis

Statistical analysis was performed using R software version 3.5.2. (2018-12-20) “Eggshell Igloo” (Oksanen et al. 2016, Team 2016). Data were tested for normality and homoscedasticity at each time point using Levene’s test for homogeneity of variance and

Shapiro-Wilk's test for normality (Brown and Forsythe 1974, Shapiro and Wilk 1965). ANOVA or Kruskal-Wallis test of ranks were performed using the R agricolae package (de Mendiburu 2016). Seedling radicle length and fungal surface area were visualized at each time point measured, and analyzed at peak growth days 35, and 49 or 60 by one-way ANOVA or Kruskal-Wallis test of ranks. Where seedling radicle length data did not display normality, data were analyzed by Kruskal-Wallis test of ranks. The Kruskal-Wallis test of ranks is a non-parametric method that tests whether samples originate from the same distribution, also called a one-way ANOVA on ranks.

3.3 Results

'Hartig Net' structures could be seen under confocal microscopy in *P. banksiana* root tips at all levels of biochar application in inoculated microcosms after 60 days incubation. This result was interpreted as confirmation that ectomycorrhizal symbiosis had been established. Other structures indicative of ectomycorrhizal symbiosis, including a fungal mantle surrounding the root tip, and intercellular hyphal clamps, were observed (Figure 3.2).

Biochar had a significant positive effect on ectomycorrhizal pine radicle length, and radicles amended with biochar reached peak length faster than those without biochar (Table 3.1). Ectomycorrhizal radicles in low biochar reached a peak mean length of 6.26 ± 0.14 cm at day 42, while radicles in microcosms without biochar had a mean peak length of 5.02 ± 0.13 cm by day 49 (Figure 3.3 A, B). Radicles amended with high biochar reached peak mean length, 6.00 ± 0.16 cm, at day 42 consistent with radicles amended with low biochar application, however growth was not increased with the higher level of biochar.

Low biochar had no significant effect on the peak mycelial area of *S. tomentosus* compared to microcosms amended without biochar, while high biochar application had a negative effect on peak mycelial area (Table 3.1). As well, high biochar delayed fungal growth; peak mycelial area with no and low biochar was reached at day 49, while peak mycelial area was reached at day 60 with high biochar (Figure 3.4). Peak mycelial areas with no and low biochar were 33.8 ± 0.44 cm^2 and 36.36 ± 0.42 cm^2, respectively, at day 49. Peak mycelial area with high biochar application was 32.64 ± 0.48 cm^2, after 60 days of incubation.

Biochar did not have a significant effect on radicle growth in non-ectomycorrhizal, (non-ECM), *P. banksiana*. Non-ECM *P. banksiana* radicles reached peak length after 35 days of incubation, faster on average compared to ECM *P. banksiana* radicles, which reached peak average length at day 42. However, despite reaching peak radicle length faster, non-ECM *P. banksiana* radicles were shorter than biochar-amended ECM radicles. Non-ECM peak radicle lengths with no or low biochar amendment were 5.45 ± 0.06 cm and 5.48 ± 0.06 cm, respectively, at day 35, and peak length with high biochar was 5.33 ± 0.03 cm at day 49 (Figure 3.3 A).

Delayed growth associated with high biochar was observed in both *S. tomentosus* mycelial area and non-ectomycorrhizal *P. banksiana* radicle length. High biochar delayed mycelial growth to peak area by 11 days compared to low biochar (Figure 3.4 A), and high biochar delayed non-ECM radicle growth to peak length by 14 days compared to low biochar (Figure 3.3 A).

Biochar had a significant negative effect on *Suillus tomentosus* mycelial area in microcosms without pine (non-ectomycorrhizal *S. tomentosus*). Unlike ectomycorrhizal *S.*

tomentosus, both low and high biochar decreased fungal growth compared to non-ECM fungus without biochar. The lowest fungal area was observed with low biochar, which reached a peak mycelial area of 10.2 ± 1.36 cm^2 compared to 32.3 ± 1.12 cm^2 peak mean area in microcosms without biochar (Figure 3.4 A, B).

3.4 Discussion

P. banksiana and *S. tomentosus* in microcosm formed symbiotic ectomycorrhizas, evidenced by microscopic detection of Hartig net structures. There were no visual microscopic differences in symbiosis structures with different levels of biochar amendment; however, the presence of biochar influenced the growth rate of both partners.

Biochar amendment had no effect on radicle lengths of non-mycorrhizal *P. banksiana*. This suggests that biochar does not limit the nutrient availability from the media to seedlings. Mean radicle length remained relatively constant day 35 – 60, possibly indicating that pine growth in the microcosm had been limited by space in the microcosm, water/nutrient availability, or both. Peak radicle length was reached faster in non-mycorrhizal pines, day 35 compared to day 42 in ectomycorrhizal seedlings, possibly due to the absence of competition for resources in microcosms from a growing fungal mycelium.

Low levels of biochar increased ectomycorrhizal pine radicle lengths and rates of growth over the 60 day incubation period. This increase may be due to altered signaling between mutualistic partners, rather than a biochar-mediated change in nutrient availability, given that there was no effect of biochar on non-ectomycorrhizal pine radicles. Decreased radicle length and fungal surface area after day 42/49 in the microcosms suggests that after this point, water and nutrient availability from the agar gel could have become limited through uptake by either species.

Slower establishment of mycorrhizae, or less effective symbiotic exchange may have contributed to lower radicle lengths with high biochar amendment. High biochar amendment may have altered symbiotic function between *S. tomentosus* and *P. banksiana* by adsorbing signaling molecules, or by influencing the signals each symbiotic partner was releasing (Warnock et al. 2007, Garcia et al. 2015). Adsorption of non-polar organic molecules is a typical characteristic of PyC in soils (Preston and Schmidt 2006). High biochar amendment in the current study may elicit a similar effect as observed by Masiello *et al.* (2013), where biochar amendment had a direct inhibitory effect on soil bacterial gene expression through adsorption of acyl-homoserine lactones used in intercellular signaling.

Ectomycorrhizal symbiosis with *P. banksiana* allowed *S. tomentosus* to overcome the negative effect of biochar on mycelial growth. When ECM mycelial growth is considered over the length of the study, (60 days), we can see that *Suillus* in the absence of biochar and with Low biochar amendment gains area until peaking at day 49, while *Suillus* with High biochar amendment gains area steadily until day 60. By day 60, *Suillus* in the absence of biochar and with Low biochar amendment had lost area, suggesting that microcosm water and nutrients had been exhausted by the growing ectomycorrhizal pine after day 49. We observed that between day 49 and 60, ectomycorrhizal *S. tomentosus* retained more area with Low biochar amendment than in the absence of biochar. This observation may be attributed to increased effectiveness of ectomycorrhizal symbiosis with low biochar application, or to increased water holding capacity in microcosm gel with biochar amendment. Especially with Low biochar application, the particularly negative effect of biochar on growth observed in non-ectomycorrhizal *S. tomentosus* is overcome by symbiosis with *P. banksiana*.

It is possible that microcosms with biochar amendment more closely simulate natural conditions compared to growth media alone, and slow ECM growth to a natural rate without a symbiotic host plant. The negative effect of biochar application on non-ECM *S. tomentosus* may be explained by the observation that ECM fungi in general have lower survival without a host (Amaranthus and Perry 1987, Allen 1991). However, the stronger negative effect of low biochar amendment compared to high biochar amendment cannot be accounted for through this observation. The negative effects of biochar amendment on non-mycorrhizal fungal growth observed in this microcosm study present an opportunity for further investigation with *S. tomentosus* and other ECM fungal species to better understand the mechanisms of PyC on growth, as well as the effect of biochar surface area on mycelial growth.

In pot experiments using sub-boreal forest soils typical of northern regions of western Canada, biochar amendment (5 or 10% of soil weight) increased the abundance of *Suillus sp.* in indigenous ECM communities, whereas *Suillus* species were absent from seedling root tips without biochar amendment. These results suggest that this is a potentially fire adapted fungal species, absent from pine associations without the presence of charcoal/biochar (Robertson et al. 2012). The positive effect of biochar on ECM-*Suillus* growth in this microcosm study supports the suggested fire adaptation of this species in the presence of pine, but the profound negative effect of biochar amendment on non-ECM fungal growth emphasizes the need for an ectomycorrhizal host for success of *S. tomentosus* after fire.

Wood samples used in this project were charred for 2 hours at 500 ºC, creating biochar with 1.1 m^2/g surface area. This surface area is unusual compared to other biochars and wildfire charcoals. For example, wood samples pyrolyzed at 500 ºC for 10 hours can have between 58 and 103 m^2/g surface area, (Jindo et al. 2014). Wildfire produced pine charcoals can have surface areas between 142 m^2/g and 159 m^2/g (Hille and den Ouden 2005) and as high as 214 m^2/g (Brown et al. 2006). However, Brown *et al.* did find biochars with surface areas between 1.3 m^2/g and 3.94 m^2/g when pine samples were produced with slow/low temperature ramp rates (30 – 200 ºC/h). Temperature ramping rate was not captured in the biochar production methodology used in this project; however, a slow/low temperature ramping rate may explain the resulting surface area.

3.5 Conclusions

This study of early establishment of ectomycorrhizal symbiosis shows that biochar can enhance early growth of symbiotic partners *P. banksiana* and *S. tomentosus*. This study also showed that biochar amendment, particularly at the lower level used here, has negative effects on the growth of non-mycorrhizal *S. tomentosus*. Ectomycorrhizal symbiosis was able to overcome the negative effect of low level biochar amendment on fungal growth. The different responses of mycorrhizal and non-mycorrhizal mycelial growth to biochar amendment suggest that ectomycorrhizal symbiosis improves the survival of both symbiotic partners, not limited to the plant host.

Table 3.1. Growth of ectomycorrhizal *Pinus banksiana* and *Suillus tomentosus* in microcosms amended with biochar. Lowercase letters denote significant differences among biochar treatments using Kruskal-Wallis test of ranks.

Day	Biochar Amendment Level	Microcosm Count	Mean Radicle Length (cm)	Mean Mycelial Area (cm^2)
28	0	25	4.02 ± 0.12	16.60 ± 0.32
	Low	27	4.94 ± 0.12	16.70 ± 0.31
	High	25	5.17 ± 0.12	13.80 ± 0.32
35	0	25	4.56 ± 0.14	24.80 ± 0.63
	Low	26	5.85 ± 0.14	27.00 ± 0.62
	High	23	5.68 ± 0.15	20.80 ± 0.66
42	0	22	4.96 ± 0.15^{b}	32.10 ± 0.58
	Low	23	6.27 ± 0.14^{a}	34.70 ± 0.57
	High	18	6.01 ± 0.16^{ab}	29.20 ± 0.64
49	0	22	5.03 ± 0.13	33.80 ± 0.44^{a}
	Low	24	6.11 ± 0.12	36.40 ± 0.42^{a}
	High	18	5.96 ± 0.14	32.30 ± 0.48^{b}
60	0	22	4.69 ± 0.11	29.50 ± 0.45
	Low	23	5.67 ± 0.11	33.50 ± 0.44
	High	18	4.82 ± 0.12	32.60 ± 0.48

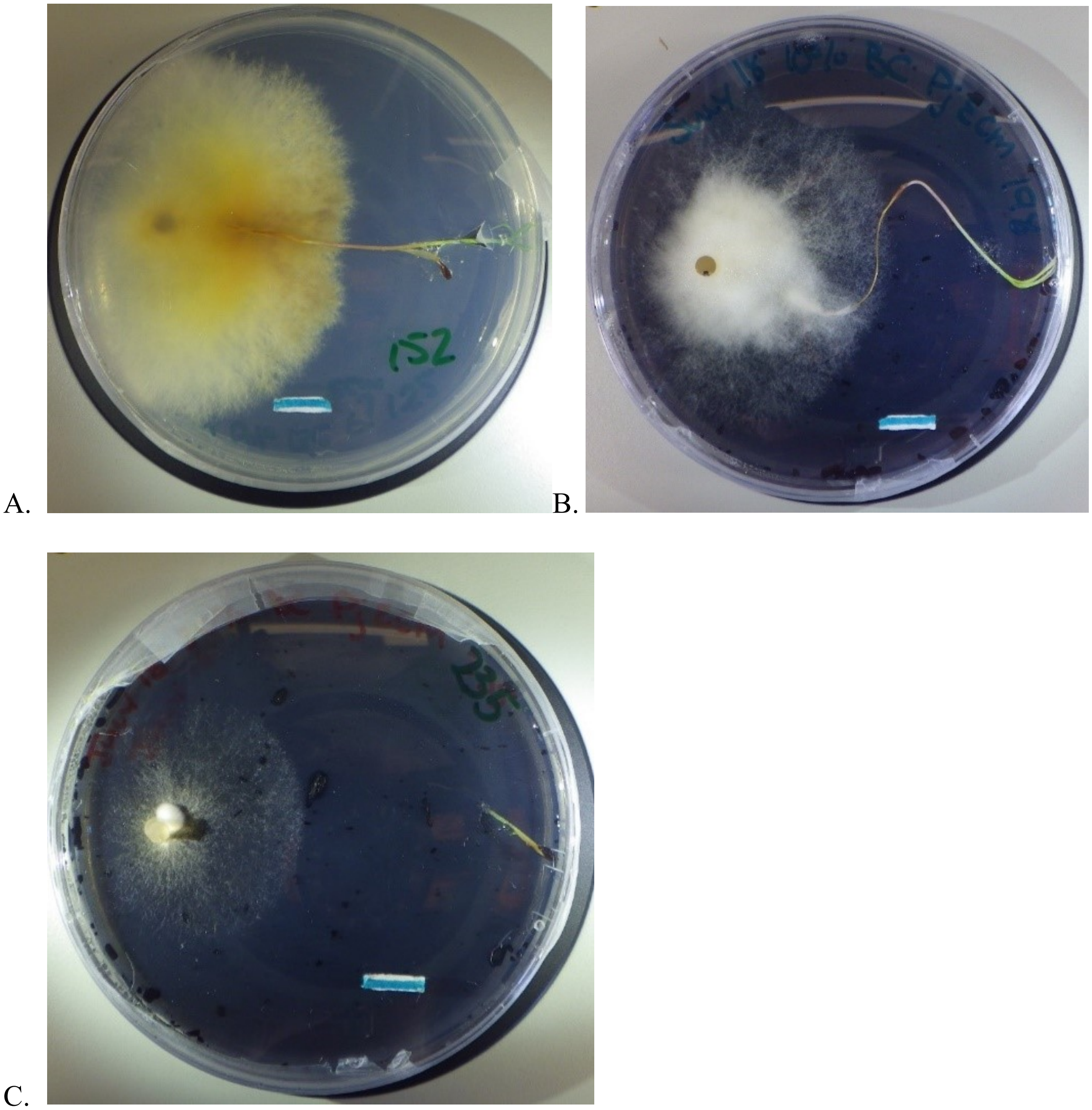


Figure 3.1. Microcosms containing *Suillus tomentosus* and *Pinus banksiana*. Photographs show three biochar amendment levels; a) no biochar, b) low biochar, and c) high biochar. Microcosms pictured are at day 35.

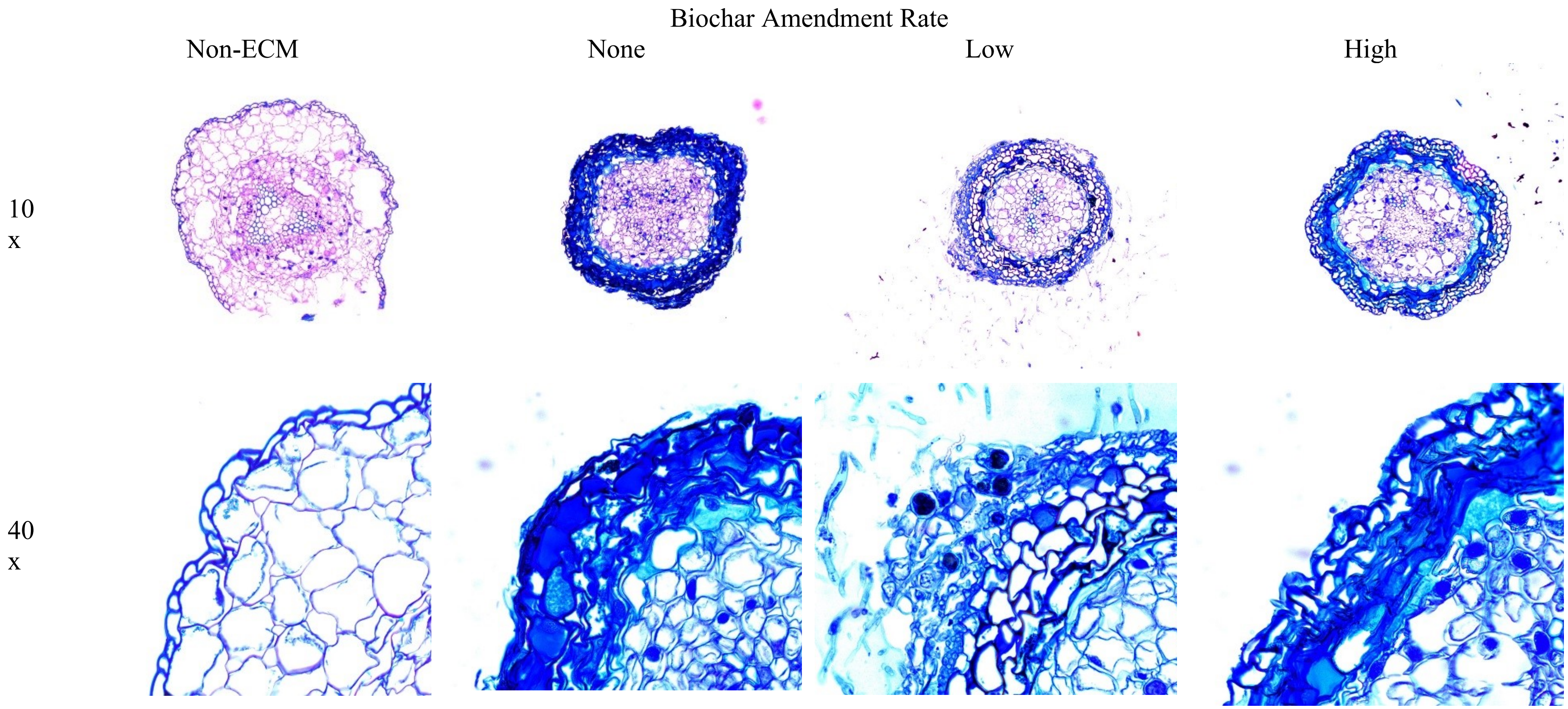


Figure 3.2. *Pinus banksiana* root tip cross sections. Cross sections of root tips show structures indicative of ectomycorrhizal symbiosis with *S. tomentosus*; a non-ectomycorrhizal (non-ECM) is included for comparison. 8 µm sections were embedded in wax and stained with toluidine blue; both fungal and plant root cells appear blue. Images were prepared at ten and forty magnification power.

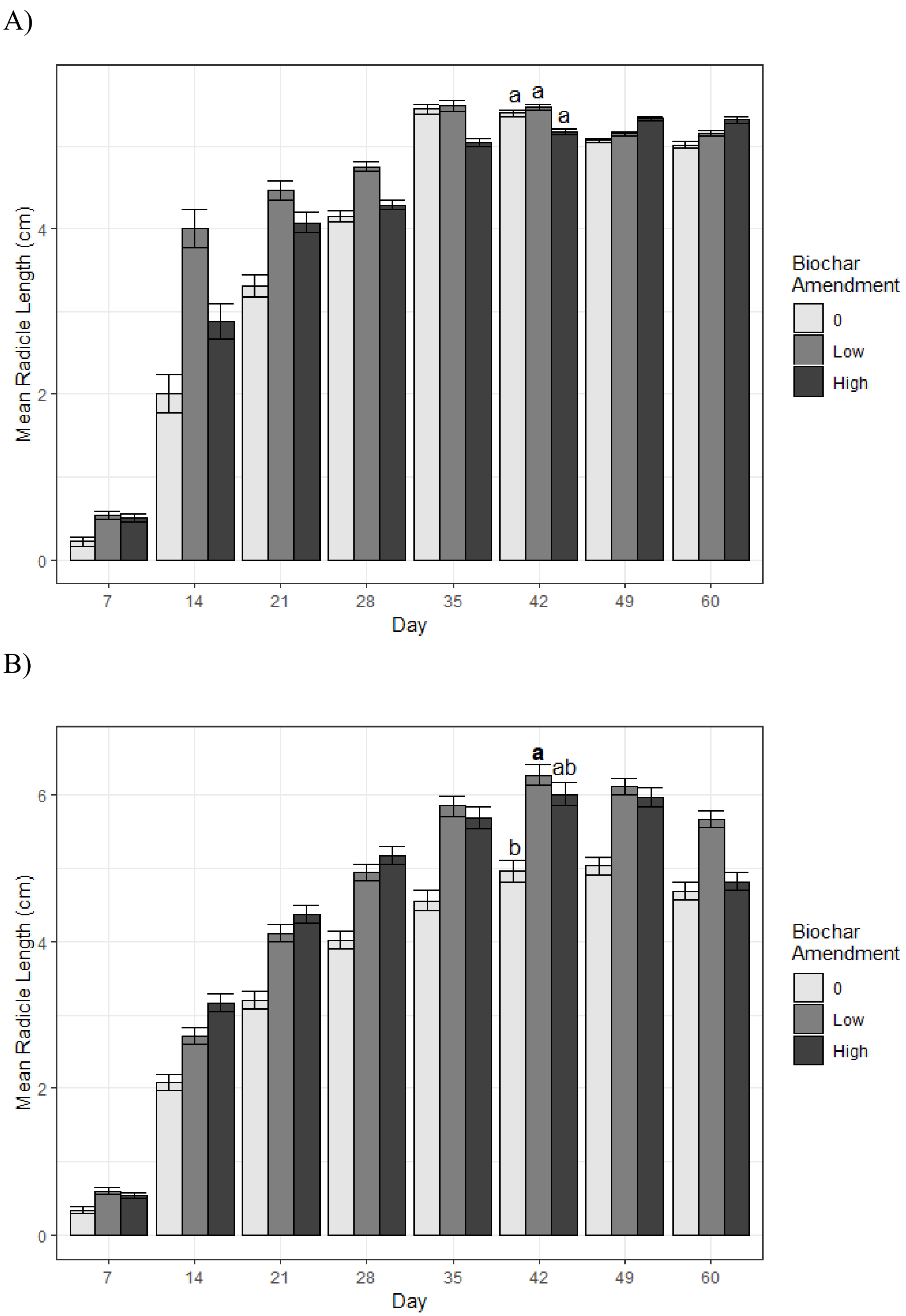


Figure 3.3. The effect of biochar amendment on *Pinus banksiana* radicle length. Biochar had no effect on the growth of non-mycorrhizal pine radicles (Figure 3.3 A).. Low biochar amendment has a significant positive effect on mycorrhizal pine radicle length (Figure 3.3 B).

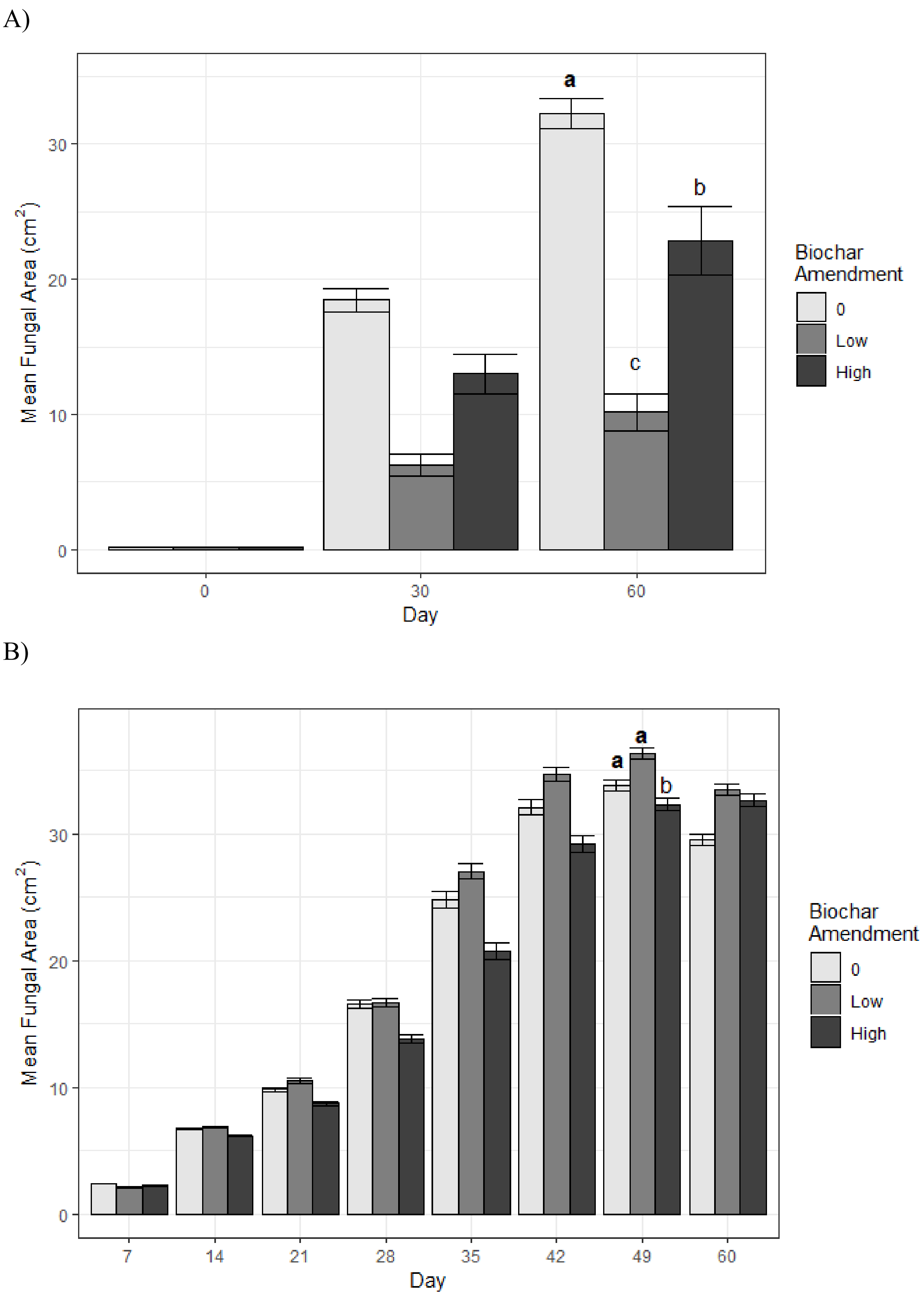


Figure 3.4. The effect of biochar amendment on *Suillus tomentosus* mycelial area. Biochar amendment had a negative effect on non-mycorrhizal *S. tomentosus* mycelial growth (Figure 3.4

A). Mycelial area was analyzed by Kruskal-Wallis test of ranks after 60 days in microcosm. Biochar amendment had a positive effect on mycorrihazl *S. tomentosus* mycelial area at day 49 (Figure 3.4 B).

Chapter 4: Conclusion

This thesis investigated pyrogenic carbon (PyC) properties (quantity and thermal stability) in Rocky Mountain soil and its effect on the microbial and plant community; specifically, on microbial community physiology and early ectomycorrhizal symbioses. Study data were collected from both field sampling within Kootenay National Park (KNP) burn sites and from microcosms grown in a growth cabinet at the University of Alberta. PyC was quantified in sampled soils using benzene polycarboxylic acids as molecular markers. Soil organic matter (SOM) quality was characterized using simultaneous thermal analysis which allowed description of heat of combustion evolution as it relates to PyC recalcitrance, and physiological profiles of the soil microbial communities were established from respiration responses to organic substrates. Following burn site soil analysis, a mycorrhizal fungal symbiont of pine commonly found in Rocky Mountain soils, *Suillus tomentosus*, was grown in PyC amended microcosms with *Pinus banksiana*.

The objective of burn site soil sampling and analysis was to gain insight on the effectiveness of prescribed burning at mimicking the soil conditions created by wildfire in KNP. While sampling design limitations, discussed further below, reduced the scope of interpretation available from that data, the study found differences between the prescribed burn site and wildfire sites within the studied parameters. The PyC content within an area of the prescribed burn site was lower than PyC found at the wildfire sites, which may suggest that on average this area burned with less intensity. A greater amount of SOM was also found within the prescribed burn site compared to the wildfire sites, which was also potentially indicative of lower combustion efficiency associated with lower fire intensity. Finally, the soil microbial community physiology at the prescribed burn site was distinct from the wildfire sites, pointing to a possible influence from PyC and SOM. The PyC within the 2003 wildfire burned area in Vermilion Pass was comparable to published values from an intense wildfire in Yellowstone National Park (Tinker and Knight 2000).

Building on the comparison of wildfire and prescribed burn soils, the second objective of this thesis was to investigate how differing quantities of PyC affect the formation of ectomycorrhizal symbioses and growth of ectomycorrhizal pine. Microcosm PyC amendment levels were most similar to the amounts of PyC quantified in soils from the 2003 wildfire, 5168 kg/ha, and the fireguard burn, 3686 kg/ha, in KNP. PyC had a positive effect on growth of ectomycorrhizal *Pinus banksiana* and *Suillus tomentosus* at both amendment levels, with significantly more growth observed at the lower amendment level. Inversely, PyC was shown to negatively affect mycelial growth of non-mycorrhizal *S. tomentosus* at both amendment levels, with significantly less growth observed at the lower amendment level. Numerous factors may be implicated in these inverse responses, regardless, the advantage of symbiosis for fungal survival in the presence of PyC is clear.

4.1 Study Limitations

The burn site and sampling design used to collect soil data was limited in two ways. First, despite both the 1968 and 2003 burn sites in Vermilion Pass being created by wildfire, they could not be treated as replicates due to natural variability in fire behavior and burning conditions. Second, the sampling design, three 100 m transects 10 m apart horizontally across the burn, captured a small area within each burn, so limited the scope of interpretation available from the data. These study limitations restricted the type of statistical analysis available to the dataset, meaning that statistical significance could not be assigned to pyrogenic carbon content based on type of fire (wildfire or prescribed burn) through parametric techniques, for example by analysis of variance (ANOVA). Because this sampling design captured a small area within large burns the spatial scope of interpretation was limited to conclusions about local conditions, rather than wider interpretations about the ability of prescribed burning to create soil conditions similar to wildfires in this region. A larger representation of each site could have been captured by placing transects or circular plots randomly throughout each site, rather than together in one area of the burn. Further, sampling a larger number of burn sites throughout Kootenay National Park would add valuable scope of interpretation and understanding of soil conditions created by fire throughout this landscape.

Ectomycorrhizal (ECM) symbioses were successfully established in microcosms, and PyC had a positive effect on growth of both *P. banksiana* and *S. tomentosus* when an ectomycorrhizal symbiosis was intact. However, microcosms are an artificial system that exclude bacteria and the study design only followed early ECM establishment and growth. Therefore, it is not clear from this study that the positive effects on PyC on ECM establishment and growth will translate into a long-term advantage for either pine or fungi. The experiment could be stepped out into pots and the growth of ECM symbionts monitored for a longer period of time to determine if the positive effects on early growth imparted by PyC increase seedling survival. Unexpectedly, PyC had a negative effect on *S. tomentosus* alone. This observation opens a line of investigation into the mechanism responsible for this negative effect.

4.2 Fire Restoration in Kootenay National Park

The wildfire and fireguard burn sites studied here are located at the northern termination of Kootenay National Park's Vermilion valley. This is the connection point with Banff National

Park and the more highly populated Bow valley. The 2003 wildfire and fireguard study sites burned during an exceptionally active fire season that lead to the implementation of a strategic prescribed burn plan that would allow fire to return with a closer frequency to its historic regime (Derworiz 2013). Kootenay National Park faced another exceptional wildfire season in 2017, with the Verdant Creek wildfire burning more than 17,644 hectares (Parks Canada Agency 2018). Any effort to restore fire to a more natural role in large wilderness areas like the national parks must recognize the paradox that suppression of natural fires has greatly altered fire frequency and fire severity, important processes that historically shaped wilderness ecosystems (Arno et al. 2000). Fire exclusion has created conditions for modern fires that are larger, more intense, and more severe because of high forest biomass load, multi-layer canopies, and fuel continuity across the landscape (Keane et al. 2003).

While prescribed burning is able to mitigate extreme wildfire behavior and severe fire effects (Cochrane et al. 2012), large wildfires like Verdant Creek 2017 highlight the challenges faced by land managers in implementing prescribed fire programs that are able to effectively counteract the fuel build-up and continuity created by fire exclusion. Little is known about the relative impact of prescribed fire in actually mitigating effects of high intensity wildfire on soils. The important ecological role of frequent, low intensity fires includes the creation of both fire-adapted plant and soil microbial communities, which impart long-term resilience to natural and anthropogenic disturbance (Choromanska and Deluca, 2001). Gray and Kubian (2010) noted the need for repeated prescribed burns in stands with high fuel loads, and recommended burn prescriptions that gradually reduce large-fuel loading over a series of burns.

Prescribed burning programs in ecosystems with continuous heavy fuels must balance the benefits of fire restoration with the risk that escaped fires will spread quickly across these large landscapes (Ryan et al. 2013). Therefore, prescription burns are planned conservatively, requiring fuel moisture, wind speeds, and relative humidity that minimize the chance of fire escape. Unfortunately this narrow set of acceptable conditions is uncommon, meaning some years have only a few appropriate days for burning (Quinn-Davidson and Varner 2012). The availability of resources and cost of staffing and equipping a prescribed burn team further challenge prescribed burning programs to replicate the scale, fire heterogeneity, and fire frequency of historical Rocky Mountain fire regimes (Leenhouts 1998, Stephens et al. 2007). To off-set these challenges strategic management of wildfires will continue to be a required and more realistic option for accomplishing fire restoration that more closely replicates the regional historic fire regime (Collins et al. 2009, Ryan et al. 2013). An expanded program of soil analysis to quantify PyC and characterize its thermal stability could be a useful tool to monitor the progress of these restoration strategies.

The PyC amended ectomycorrhizal pine-fungal microcosm experiment undertaken for this thesis showed that a PyC quantity consistent with the amount deposited by moderate-low severity fires has a positive effect on the early growth of ECM pine and *Suillus* fungi. These results demonstrate that moderate severity prescribed fires may be beneficial to establishing mycorrhizal pine seedlings and play an important role in fire restoration in Rocky Mountain ecosystems. Ectomycorrhizal (ECM) fungi colonize organic and upper mineral soil horizons and are therefore sensitive to deep soil heating that results from burning thick organic layers that accumulate after years of fire suppression (Borchers and Perry 1990). Low severity fires may leave part of the organic layer unburnt and have less lethal impact on the ECM fungal community. *Suillus* inoculation has been shown to enhance pine seedling survival under harsh, competitive, conditions like those left behind after fire (Lonergan et al. 2014), and charcoal amendment was shown to have positive effects on pine shoot growth and tended towards higher numbers of ECM colonized root tips in field trials (Robertson et al. 2012). An advantage of prescribed burning under typical mild conditions could be less soil heating, leading to lower levels of fungal mortality, and less efficient combustion leading to less PyC production compared to a high severity burn under same fuel load. Results of this thesis suggest that these conditions could have a positive effect on pine regeneration and highlight the role for moderate intensity prescribed fire in fire restoration programs in the Rocky Mountains.

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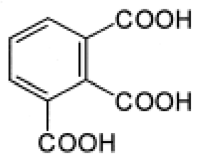
Appendices

Appendix 1: Pyrogenic carbon characteristics in soils following wildfire and prescribed fire in Kootenay National Park

1.1 Benzene polycarboxylic acid methodology for PyC quantification



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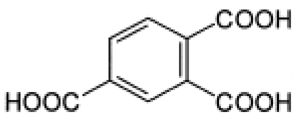

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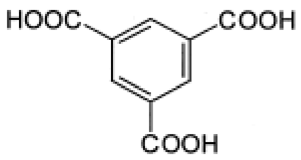

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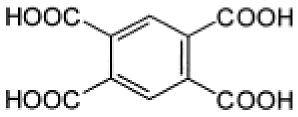

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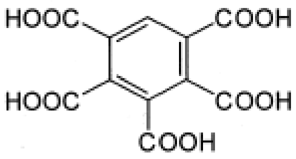

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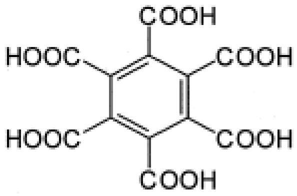


B6CA

Appendix Figure 1. Chemical structures of benzene polycarboxylic acids (BPCA) quantified as molecular markers for pyrogenic carbon.

1.2 Soil microbial community-level physiological profiles

Appendix Table 1. Community-level physiological profile substrates.

Carbohydrates	Amino Acids	Carboxylic Acids
L (+)-Arabinose *D (+)-Galactose* *D (+)-Glucose* *D (-)-Fructose* *D (+)-Trehalose 2* H_2O *Cellulose*	*γ-Aminobutyric acid* *L (+)-Arginine* *L-Lysine HCl* *L-Cysteine HCl*	*Fumaric Acid* *Malic Acid* *Oxalic Acid* *Citric Acid* *3,4-Dihydroxybenzoic Acid*

Appendix 2: Biochar amendment has positive effects on growth in *Pinus banksiana* ectomycorrhizal symbioses

2.1 Seed Surface Sterilization with Hydrogen Peroxide Methodology

Surface sterilization of the pine seeds was required to prevent growth of bacteria. This method was adapted from "Protocol for Surface Sterilizing Douglas Fir (DF) Seeds" received from J. Karst. The objective of this method optimization was to find the minimum peroxide exposure time required to eliminate bacterial contamination, while least delaying emergence of the seedling radicle.

Jack Pine seeds were soaked in room temperature tap water for 24 hours prior to exposure to hydrogen peroxide. Seeds were exposed to 50 mL of 30% hydrogen peroxide in groups of 10 in a 125 mL Erlenmeyer flask. Exposure times tested were 0 – 35 minutes, in steps of 5 minutes. Seeds were gently agitated using the bubbler set-up described in section 2.1.1 to promote even exposure to the hydrogen peroxide.

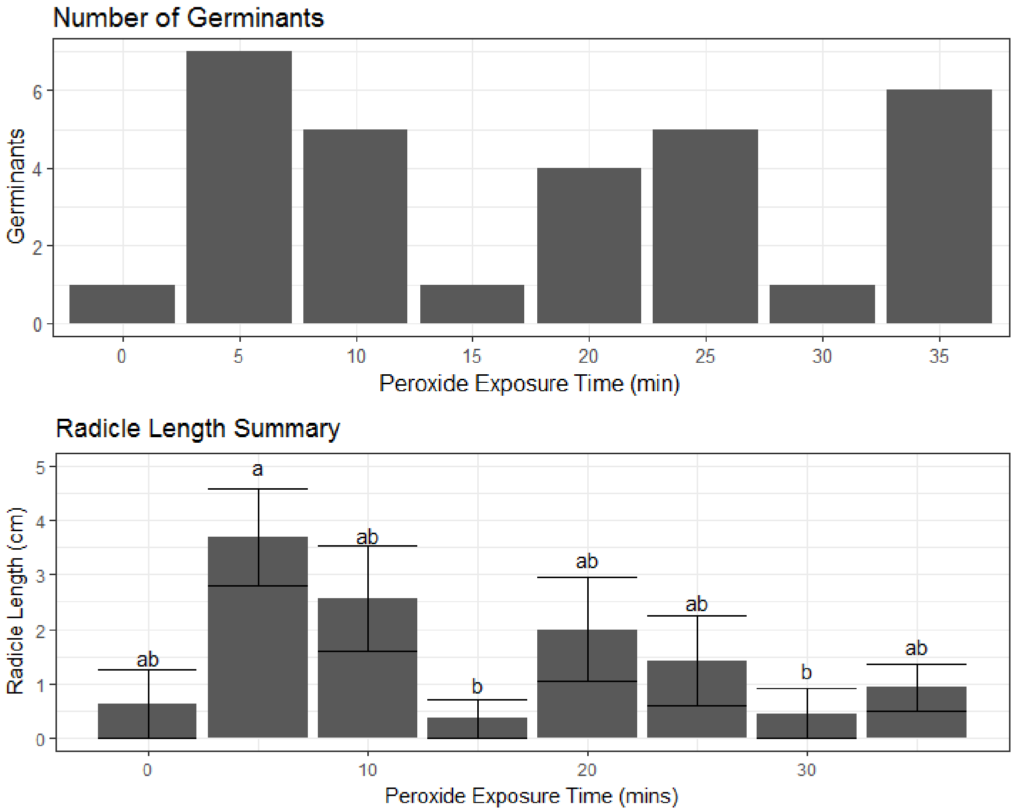
MMN media was poured 1 cm deep in 110 mm plates, as per dehydrated media package instructions. Sterile water was prepared by boiling 750 mL of tap water for 10 minutes in a 1 L Erlenmeyer flask, with foil over mouth, and cooling for minimum1 hour in a 4 ºC fridge.

Flame sterilize mouth of sterile water, pour ~80 mL into the 125 mL peroxide flask to 'quench' sterilization. Seeds float to the top of the liquid. Using flame sterilized inoculating loop, pull seed from liquid, place on prepared MMN agar plate. Cover and seal with double layer of parafilm. Allow a few minutes for the seed to sit flat on agar prior to vertical storage in plate rack. 0 min seeds were placed in 125 ml sterile water prior to plating.

The most radicles emerged from seeds in the 5 minute peroxide exposure group, 7/10, followed by 6/10 in the 35 minute exposure group (Figure 3). 35 mins was not chosen because, while surface sterilization occurred, the longer exposure time possibly delayed radicle emergence. This idea is supported by analysis of the radicle lengths (Figure 3. A1.2), where the longest radicle lengths are associated with 5 minutes exposure to hydrogen peroxide.

2.1.1 flask and bubbler set up:

Cover mouth of 125 mL Erlenmeyer flask with foil. Set 1 mL pipette tip in tubing to compressed air line. After seeds added to peroxide in flask place pipette tip in peroxide. Gently turn on air to create gentle bubbles. Foil around tubing into the flask to cover the mouth of the flask.



Appendix Figure 2. Number of seeds with radicle emergence after 14 days, 10 seeds per exposure time. Jack Pine radicle length (cm) by hydrogen peroxide exposure time (minutes).

2.2 Modified Melin-Norkrans (MMN) Preparation

Modified Melin-Norkrans (MMN) is a standard nutrient medium used for the isolation, maintenance and propagation of fungal cultures. Pre-packaged dry MMN media (bioPLUS Melin-Norkrans, modified) was used per manufacturer instruction, 20.82 g dry media per 1L of water, with 10 g of dry agar. After weighing all the dry ingredients dissolve in 1 L of ultra-pure water. Stir the media with a magnetic stir bar on stir plate. Heat media to 100 °C, then allow to cool 10 minutes on benchtop. Once cooled, add 100 µg of thiamine HCl or 3 µL of 0.1M HCl thiamine stock solution, then buffer the media to pH 5.6 using 1M HCl or 1M NaOH. Finally, add 25 µg/mL of filter sterilized streptomycin.

Appendix Table 2. Microcosm media preparation by biochar treatment level.

	0 Biochar	Low Biochar	High Biochar
MMN media prep	20.82 g	20.82 g	20.82 g
Agar	10 g	10 g	10 g
Biochar	0 g	2.08 g	4.16 g